Diapause by seed predators and parasitoids in *Chionochloa* mast seeding communities

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Ecology

November 2008

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"We will now discuss in a little more detail the struggle for existence"

(Charles Darwin, *The Origin of Species*, p.49)

Acknowledgments

This PhD thesis could not have been come to pass without the assistance of Prof. Paula Jameson who highly recommended Prof. Dave Kelly as a potential supervisor. Her advice was one of the most excellent I have been given in my life.

I would like to thank my supervisors, Prof. Dave Kelly and Dr. Ecki Brockerhoff for their excellent supervision and expertise. Without their enthusiasm, support and patience, throughout this entire project, I could not have done this. I thank both of them for the positive atmosphere, for being so approachable and friendly and for their brilliant ideas and guidance. I could not ask for better supervisors! Thanks for that!

I would also like to thank Prof. Dick Pharis for his helpful comments, suggestions and willingness to help. Dr. Matthew Turnbull, Dr. Roger Dungan, Prof. Ottar Bjørnstad, Dr. Lisa Berndt, Dr. Steve Pawson and Dr. Jason Tylianakis gave statistic and scientific help during this PhD. Prof. Alain Roques helped with midge rearing, Dr. Jo Berry (Landcare research, Auckland) and John Dugdale (Landcare research, Nelson) kindly identified the parasitoid species, Dr. Peter Kolesik described *E. chionochloae* (Chapter 2) and Dr. Peter Buhl described *Z. chionochloae* (Chapter 3), Jeanette and Alister Maxwell, Mt Hutt ski field and NZ DoC generously allowed us to work on their properties. Prof. Raymond J. Gagné (USDA, Washington DC, USA) for commenting on an early draft of the manuscript (Chapter 2), Norm Johnson (Hymenoptera on Line Database) for information on number of described platygastrid species and genera (Chapter 3)

There are an enormous number of people whom I received help from while working on this thesis:
Firstly, I thank my numerous field and lab assistances (alphabetically): Becky Bell, Brad Chandler, Veronica Clifford, Nicola Day, Emily Graham, Sabrina Luicht, James Muir, Chrissie Painting, Kate Rose, Glenn Squire and Carl Wardhaugh.

Secondly, I thank the technical and administrative help I received: (Biological Sciences) Jenny Ladley and Maggie Tisch for so much helpful advice, immense enthusiasm and energetic and fast assistance. You are awesome! I would like to thank Dave Conder, Gerald Cuthbert, Alan Lecky, Nigel Pink and Joy Write. Each of you made this thesis possible in your own individual manner. Matt Walters and Jan McKanzie helped with the brilliant insect photos!

I'd additionally like to thank my lab and office mates: Merodie Beavon, Ronny Groenteman (thanks so much for everything!), Melissa Hutchison, Rocio Jaña, Sara-Jane O'connor, Marc Merlin Thomas, Chucky van Schravendijk, Debra Wotton and Laura Young.

I thank my friends back home for their long distance endless support: Limor Herzog-Aharoni, Efrat and Barak Adani and Hila Elifantz. I also thank the wonderful friends I have met during this study; sadly, some of them are far away from me: Judit Vörös, Oz Golan, Anat Selah and Raphaela Ronen.

Finally, I thank my husband Ilan Schwartz for his love and emotional support, patience and uplifting atmosphere and to Mom, Dad, Nimrod and Yair for their unlimited support and believe I can really make it… I love you (and miss you) all very very much!

The University of Canterbury granted me with a PhD scholarship, while the project was supported by the Marsden Fund of the Royal Society of New Zealand, for which I am highly grateful.

This thesis is dedicated to my late maternal grandmother Genia Ya'acobi (Zandberg), who was the smartest woman ever.

Abstract

Chionochloa, a genus of snow tussock grasses native to New Zealand, exhibits pronounced mast seeding. *Chionochloa* suffers very high levels of pre-dispersal flower and seed predation by three main insects: *Eucalyptodiplosis chionochloae*, a cecidomyiid midge, which is formally described here; *Megacraspedus calamogonus*, a gelechiid moth and *Diplotoxa similis*, a chloropid fly.

Seven species of parasitoids that attack these seed predators were discovered. Four species parasitize *M. calamogonus* (one tachinid fly and three hymenopteran wasps), one parasitizes *D. similis* (Hymenoptera: Eulophidae) and two parasitize *E. chionochloae*, (a pteromalid wasp *Gastrancistrus* sp. and a platygastrid wasp *Zelostemma chionochloae,* which is given a formal description here).

The abundance, predation levels by each of the insect species, and interactions between all the organisms in the community were studied across three elevations at Mount Hutt over three summer seasons. *M. calamogonus* was most abundant at 450 m altitude during all three seasons. *D. similis* was most common at 1070 m altitude, while its predation levels peaked in low flowering seasons and decreased in high seasons. *E. chionochloae* was abundant in all three altitudes and increased its predation levels with increasing flowering intensity. *E. chionochloae* was confirmed to use prolonged diapause of at least three years. Prolonged diapause was also confirmed in its two parasitoids.

Chionochloa plants were manipulated with various treatments to test the effect on diapause in *E. chionochloae* and its two parasitoids. Treatments included plant warming, root pruning, gibberellic acid sprayed on the plants and combinations of these treatments. All three insects changed their emergence in response to some treatments and therefore it was suggested that combined with risk-spreading diapause, they may use some predicting to emerge from prolonged diapause. *E. chionochloae* control their diapause following some of the cues that *Chionochloa* use for flowering, while *Z. chionochloae* and *Gastrancistrus* in some cases follow their host's cues and in others use similar cues as *Chionochloa* plants. Emergence or diapause predictions differed across elevations and plant species in all three insect seed/flower predators.

E. chionochloae had female-biased sex ratios in different populations even after prolonged diapause. There was week evidence that both parasitoid species are female-biased in the first emergence year and male-biased after more than one year in diapause. Therefore it was suggested that diapause is not more costly for females of *E. chionochloae* and its parasitoid than for males. Females of all three species were not found to be better predictors (i.e, more likely to respond to treatments by not entering extended diapause) than males.

The complex interactions of all the organisms in this web are thought to be sensitive to climate, and it was suggested that the global climate change may alter this sensitive system.

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1. Introduction

1.1 Mast seeding - General

1.1.1 Introduction to Mast Seeding

The term 'mast seeding' comes from the German word for fattening livestock on abundant seed crops such as those of beech trees (Janzen, 1976; Silvertown, 1980; Kelly, 1994; Kelly & Sork, 2002). Other terms for the same phenomenon of prolific flowering can be found in the literature including gregarious flowering (Janzen, 1976), mass seeding, mass fruiting, periodic flowering, supra-annual flowering and sporadic seasonal synchrony (Kelly, 1994). Many specific definitions can be found in the literature (Janzen, 1976; Silvertown, 1980; Norton & Kelly, 1988; Webb & Kelly, 1993). Nonetheless, one of the most general definitions and the most frequently used for mast seeding is '*synchronous highly variable seed production among years by a population of plants*' (Kelly, 1994). This definition has two elements: (1) the variability of flowering intensity among years; i.e., in some years, a certain population of perennial plants will produce large seed crop while in other years seed crop will be rather small and; (2) synchrony i.e., all the individuals in the population will flower in large episodes in the same years (Kelly, 1994). The output of seed crops during years of significant flowering is variable and changes from high to low (Kelly, 1994).

The best way of measuring the intensity of mast seeding is by the coefficient of variation (CV = standard deviation/mean) of the crop size (Silvertown, 1980; Kelly, 1994). McArdle and Gaston (1995) suggested that the CV is a suitable index of proportional variability that is independent of the mean, although Buonaccorsi et al., (2003) showed that the CV is affected by other factors, such as the variability within plants, the inter-plant synchrony, plant sample size and productivity per plant. Nevertheless, it is a valuable measurement for seed output in the population level and indeed many studies used it when measuring mast seeding intensity (Silvertown, 1980; Webb & Kelly, 1993; Kelly, 1994; Herrera et al., 1998; Kelly et al., 2000; Schauber et al., 2002; Koenig et al., 2003).

Studies report mast seeding of plants from many different taxa and in boreal, temperate, subtropical and tropical climates, e.g. in temperate forest trees (Silvertown, 1980, Norton & Kelly, 1988), in tropical and subtropical areas such as Chile, China, Costa-Rica, India east through Burma, Jamaica, Japan, Madagascar and Thailand (Janzen, 1976) and in temperate grasslands, such as New Zealand (Webb & Kelly, 1993; Kelly, 1994; Kelly et al., 2000; Kelly et al., 2001), see also Figure 1c in Kelly & Sork (2002).

Because they do not reproduce every year, mast seeding plants suffer some disadvantages, such as lost opportunities for reproduction, higher density dependent mortality (e.g., in case of pathogen attack or other disasters), and lower population reproductive rates (Kelly & Sullivan, 1997; Kelly et al., 2000; Kelly et al., 2001). That is probably why mainly long-lived, perennial plants which have lots of opportunities to reproduce during their lifetime can afford to mast (Waller, 1979).

However, there must be some evolutionary advantage for the selection of mast seeding in so many species of plants. The evolutionary benefit of mast seeding is possibly explained by at least eight theories; of these, three have attracted most attention: predator satiation, wind pollination and environmental prediction (Kelly, 1994). All these theories are versions of 'economy of scale hypotheses', as suggested by Norton and Kelly (1988) where 'large episodes of reproduction are more efficient than small ones'.

1.1.2 Predator satiation

Janzen (1971) proposed the hypothesis that mast seeding behaviour in plants evolved to satiate seed predators. The theory suggests that in mast years there would be so many seeds available for seed predators that the latter would not be able to kill all the seeds before some are dispersed and grow (Janzen, 1974). Silvertown (1980) added that seed predator populations decrease in the low seed years from starvation. Therefore the interval between high flowering years should be long enough that the seed predator growth of populations due to the increase in food supply would decay before the next mast year is produced. Hence, during low flowering years, populations of seed predators would starve (Kelly, 1994). Another critical aspect for the success of the predator satiation theory is that species of plants that share the same species of seed predators should mast in synchrony (Silvertown, 1980).

The predator satiation theory is the most commonly invoked to explain mast seeding behaviour in plants (Kelly, 1994) and there are many studies which support it empirically, e.g., Shibata et al., (1998) studied mast seeding in four species of *Carpinus* trees in Japan and found clear evidence for predator satiation. Kobro et al., (2003) studied the evolutionary benefits of masting in *Sorbus aucuparia* in Norway and found that this behaviour provides an adaptive defence against the apple fruit moth *Argyresthia conjugella*, an important seed predator of *S. aucuparia*. Other studies show similar results in different species of plants and herbivores (Silvertown, 1980; Kelly & Sullivan, 1997; Rees et al., 2002; Satake & Bjornstad, 2004). However, some studies fail to find an effect, for example, Lazaro et al., (2006) tested

whether mast seeding in *Buxus balearica* shrubs found at the western Mediterranean basin evolved as a consequence of its seed predators, generalists rodents and ants, but did not find any evidence for predator satiation. However, as mast seeding is more effective against specialized, host-specific seed predators (Smith et al., 1990), it is conceivable that predator satiation did not work in this case. Nilsson and Wastljung (1987) found that the proportion seed crop of *Fagus sylvatica* beech trees destroyed by predators was larger in non-mast years than in mast years. In addition, their data also suggest that the evolutionary benefit of mast seeding in these beech trees is largely supported by the wind pollination theory.

1.1.3 Pollination efficiency

A large number of plant species that exhibit mast seeding use wind pollination for reproduction (Janzen, 1971, , 1976; Silvertown, 1980; Kelly, 1994). Wind pollinated plants that concentrate their pollen production into fewer years increases the probability of pollination (Smith et al., 1990) and, therefore, mast years will be more efficient in plant reproduction and recruitment (Nilsson & Wastljung, 1987; Norton & Kelly, 1988). In addition, because all the individuals in the population reproduce at the same time, the genetic diversity of the pollen rain distributed is very high and, therefore, cross pollination is elevated and genetic variation is higher (Houle, 1999).

Kelly et al., (2001) proposed a theoretical model which predicts whether wind pollination is efficient in different mast seeding species or not. They found that masting will be favoured in wind pollinated species that have low pollination success at the long-term-mean flowering effort.

Nilsson and Wastljung (1987) found that successful pollination and fruit set in *Fagus sylvatica* (European beech) trees were dependent on the density of flowering plants and the number of neighbours. They also found that pollination success was higher in mast years than in non-mast years. Norton and Kelly (1988) studied mast seeding in rimu (*Dacrydium cupressinum*) and found strong support for wind pollination as a selective force. Mast seeding in oaks in California efficiently increased pollination success (Koenig et al., 1994). On the contrary, Kelly and Sullivan (1997) studied the evolutionary benefit of mast seeding in *Chionochloa pallens* and concluded that wind pollination cannot explain the evolution of mast seeding in *C. pallens*.

To summarize, different plants increase their pollination efficiency by wind pollination at different levels. In other words, mast seeding was selected in some plant species because wind pollination efficiency increased their fitness; in other plant species mast seeding was selected because of other factors such as predator satiation while there are plant species that show a combination of few factors that selected for mast seeding.

1.2 Mast seeding in New Zealand

Many species of plants from many families and taxa show mast seeding in New Zealand flora (Norton & Kelly, 1988). These species grow in a wide range of altitudes and habitats and pollinate and disperse their seeds in different ways (Webb & Kelly, 1993).

In general, mast seeding is more extreme at higher elevations (Webb & Kelly, 1993), and climate has a great influence on the timing of mast years (Schauber et al., 2002). Moreover, because most plants use weather cues to time masting, even plants from different families (e.g. *Chionochloa*, *Phormium* and *Nothofagus*) are often synchronized and flower at the same time (Schauber et al., 2002).

1.2.1 Mast seeding in *Chionochloa*

Chionochloa is a genus of snow tussock grasses with 23 species endemic to New Zealand, one species (*C. frigida*) that is endemic to Australia, and another species (*C. howensis*) that is endemic to Lord Howe Island (Connor & Lloyd, 2004). Species of the genus *Chionochloa* are important perennial long-lived snow tussock grasses (Connor, 1967).

In the 1960's Alan Mark and Henry Connor studied infrequent flowering in *Chionochloa*, which was found to be related to high temperatures during the summer season of the previous year (Mark, 1965c, Connor, 1967). In addition, Mark (1965a) noted that not only temperature and day length are critical for the flowering of *Chionochloa* but also the energy reserves the plants possess. Plants with depleted resources could not flower even if they had been exposed to high summer temperature in the previous year. Furthermore, Mark (1965b) showed that the flowering of *C. rigida* synchronizes between individuals in accordance to temperature. Later, Kelly et al., (2000) listed 11 different species of *Chionochloa* which show different degrees of masting.

Plants at different elevations synchronize according to the deviations in local expected temperature cues, not absolute temperature (Schauber et al., 2002). Therefore, flowering synchronizes over a very large scale measured in hundreds or thousands of kilometres (Kelly et al., 2000; Kelly & Sork, 2002). *Chionochloa* species have high CV of flower production relative to other grasses, shrubs or trees reported in the world (Kelly & Sullivan, 1997; Kelly et al., 2000). *Chionochloa* CVs range from 1.42 in *C. macra*, which is the lowest variant in flower production from *Chionochloa* species (but still relatively high compared with other plant species), to 3.02 in *C. crassiuscula* that is amongst highest variant in flower production worldwide (Kelly et al., 2000; Kelly & Sork, 2002). *Chionochloa* flowers are wind pollinated (Kelly & Sullivan, 1997; Tisch & Kelly, 1998; Kelly et al., 2000; Kelly et al., 2001) and their seeds disperse by gravity (there would be short-range wind dispersal) (Kelly et al., 2000). However, as already mentioned, Kelly and Sullivan (1997) and Kelly et al., (2001) studied wind pollination in *Chionochloa pallens* and found it provides very little selective benefit from mast seeding in these species.

Chionochloa suffer very high levels of pre-dispersal seed predation (Kelly & Sullivan, 1997; Sullivan & Kelly, 2000). Mark (1968) was the first to suggest that mast seeding in *Chionochloa* originated in order to satiate these seed predators. Kelly et al., (1992) and Schauber et al., (2002) suggested that all *Chionochloa* species might share the same seed predators. Indeed a later study showed that all *Chionochloa* species, which were examined, were found to be preyed on by orange larvae of a single undescribed cecidomyiid species with no evidence for insect speciation by host plant species (McCall et al., 2004).

Burrows (1961) and Mark (1965a; 1965c) first found orange larvae of the cecidomyiid midge feeding on the ripening seed. Later on, White (1975) investigated damage to *Chionochloa* florets and reported a gelechiid moth (*M. calamogonus* Meyrick) and a chloropid fly (*D. similis* sp.) later described by Spencer (1977) as *D. similis* Spencer. McKone et al., (2001) summarized the biology and ecology of these three insects, including their geographical distributions.

The behaviour of mast seeding in *Chionochloa pallens* was found to be mathematically chaotic (Rees et al., 2002). Kelly et al., (2000) and McKone et al., (2001) attributed the chaotic behaviour of *Chionochloa* to the heavy seed predation by the three seed predators, which cause great loss of intact seeds, but especially to the undescribed cecidomyiid, which

was thought to probably use sophisticated life history strategies (i.e., prolonged diapause) to skip the low flowering years.

1.3 *Chionochloa* morphology and the seed/flower predators

Chionochloa exhibits typical grass (Poaceae) morphology: the *inflorescences* are special branches which grow from the centre of the base of tussocks. The number of inflorescences per tussock is highly variable among years, species and elevations (Kelly et al., 1992) and is basically the unit by which scientists usually measure the intensity of flowering. Each inflorescence has *spikelets* on opposite sides along the branch. At the bottom of the spikelets there are two *glumes* which are empty scales that protect the immature spikelet. *Lemma* and *palea* together enclose a set of floral organs, which are called *florets.* Every floret contains three *stamens* (anthers) and an ovary with two plumose *stigmas* (Clayton & Renvoize, 1986) (Figure 1.1). The number of spikelets per inflorescence and the number of florets per spikelet do not vary much between years (mean number of spikelets per inflorescence and florets per spikelet in *Chionochloa pallens* from 1986 to 1992 were 40.5 and 5.18 respectively) (Kelly et al., 1992).

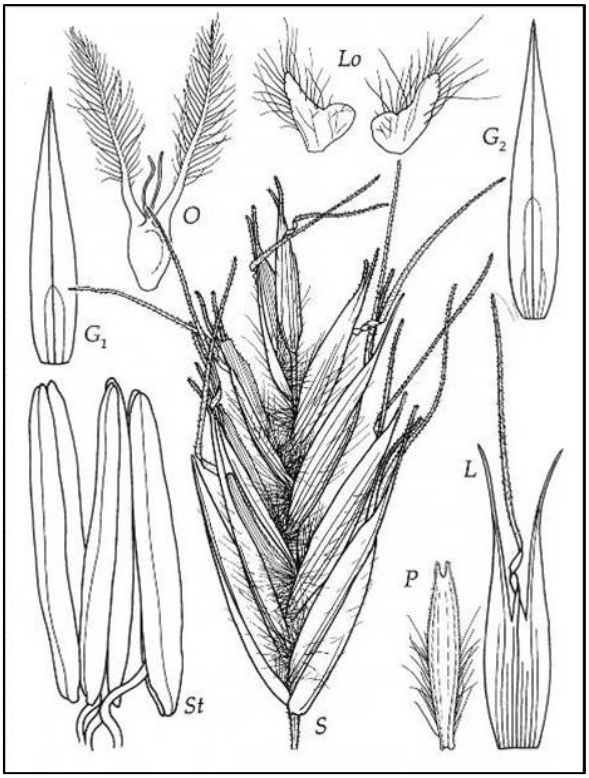

Figure 1. 1 Parts of *Chionochloa rigida* and the terminology employed. S, Spikelet; G1 and G2, Glumes; L, Lemma, dorsal view; P, Palea, dorsal view; Lo, Lodicules; O Ovary, styles and stigmas; St, Stamens (anthers) (from (Clayton & Renvoize, 1986).

The three plant species of *Chionochloa* I worked with are *Chionochloa rubra*, *C. pallens* and *C. macra*. See Table 1.1 for differences in the biology and physiology of the three species.

Table 1.1 Main biological and physiological comparisons between three *Chionochloa* species, information summarized Connor, 1991

Chionochloa sp.	Sub-species	General	Altitude	Distribution	Sizes				
					Glumes	Lemma	Palea	Anthers	Ovary
rubra	spp. *cuprea*	Tall, slender red tussock with crowded, erect, stiff junceous leaves	See level to 1500 m	South Island: from Okuku, Canterbury south to Stewart Island and west to Fordland.	12 – 14 mm	6 mm	8 mm	5 mm	1 mm
pallens	spp. *pilosa*	Tall, pale tussock with inflorescences pink-tinged.	To 1650 m but higher altitudes in Nelson	South Island: Mountain ranges in southern Marlborough and Nelson, North Canterbury and Westland	11 - 14 mm	6 mm	8 mm	5 mm	1 mm
macra		Modest size with persistent sheaths and soft laminae weathering in situ.	500-1700 m; at lower altitudes present on shaded aspects of rolling terrain.	South Island: east to main divide from Southern Marlborough to Southland.	13 mm	5 mm	7.5 mm	4 mm	1 mm

As *Chionochloa* has strong mast seeding, its seed predators face an unreliable food supply. They would be expected to deal with this in one of two ways: feed on alternative host plants, or have extended diapause to be able to wait out low-flowering years. The three common *Chionochloa* seed predators seem to span both these strategies.

1.3.1 *Diplotoxa similis*

From McKone et al., (2001) we know that eggs of *D. similis* are relatively large (0.5–0.9 mm) (compared with those of the other insects present in *Chionochloa* spikelets), white and elongated, usually laid within the glumes of the spikelets in early December and hatch in early January. Larvae feed on both stamens and pistils before anthesis and form a dark puparium in the florets when florets mature usually around mid-January. Adults emerge in February, late in the flowering season, and probably over-winter until mating and oviposition take place in late November/early December (See Figure 1a, b and c in McKone et al., (2001)).

Cone (1995) found a single *D. similis* adult in a bristle tussock (*Rytidopsperma*) floret at 1000 m, Mt Hutt, and also characteristic damage signs on the anthers (p.84). That suggests that *D. similis* might also feed on *Rytidopsperma* which could help it survive low-*Chionochloa*-flowering years. In addition, two adults from a different species of the same genus, *D. similis moorei*, were found in different dates inside nests of the ant *Monomorium integrum* Ford at Mt Grey valley, Canterbury and at Molesworth (Marlborough), 915 m elevation (Spencer, 1977). It is not clear whether these larvae were parasitic or scavengers or whether other species of the genus use this technique as a strategy for surviving low flowering years.

1.3.2 *Megacraspedus calamogonus*

Eggs of *M. calamogonus* have never been positively identified in the literature, but its larvae are 0.6–0.8 mm long and pale with reddish longitudinal stripes, (Cone, 1995; McKone et al., 2001). Their brown pupae are not normally associated with the florets and it is thought that they may drop to the ground to pupate in the soil. Adults emerge at the end of January (See Figure 4 in McKone et al., (2001)).

M. calamogonus larvae is known to have two hymenopteran parasitoid species from two different families (White, 1975; Cone, 1995; McKone et al., 2001), although only one of these has been described (Berry, 1999). Like *D. similis*, adult *M. calamogonus* are presumed to

over-winter until the following spring, when mating and oviposition occur. *M. calamogonus* is highly synchronised with *Chionochloa* flowering, and it feeds on floral parts early in the flowering season (McKone et al., 2001). Hudson (1928) found *M. calamogonus* feeding on toetoe (*Cortaderia*) in March and mid-winter, and White (1964; 1975) reported five adults in August within the base of *Poa cita*. It is not clear whether *M. calamogonus* actually feeds on these plant hosts or whether adults just hide there over winter, but *Cortaderia* florets are very similar to those of *Chionochloa* in both floret structure, arrangement of florets on the inflorescence, and general size of the floral parts (see Connor and Edgar 1974). According to Connor and Edgar (1974) and Clayton and Renvoize (1986), *Chionochloa* species share many similarities with *Cortaderia* and *Rytidiosperma* species: One species, now called *Cortaderia quila*, was mistakenly considered as *Chionochloa conspicua* but later was separated out to different species and genera. Therefore it is plausible that the moth could feed on both genera. The florets of *Poa* are rather different in size and arrangement on the inflorescence (Clayton and Renvoize, 1986) , so it seems less likely that M. calamagonus feeds on this species (as opposed to using it as a pupation site).

1.3.3 *Eucalyptodiplosis chionochloae* (Previously "The undescribed cecidomyiid")

The recently described *Eucalyptodiplosis chionochloae* (Kolesik et al., 2007) (Chapter 2 in this thesis) was known only as larvae for many years. Larvae have been collected throughout the South Island in tussocklands as well as on Mt Taranaki and the Volcanic Plateau in the North Island (McKone et al., 2001). The mobile, partially orange first instars hatch within days of oviposition in early January, and feed on developing seeds. Adults have a black head, an orange-black striped thorax and a bright orange abdomen (See Figure 2.4). Different studies suggested that the undescribed cecidomyiid is capable of entering prolonged diapause, which helps it to survive low flowering years and emerge during high flowering years (Kelly et al., 2000; McKone et al., 2001; Rees et al., 2002).

1.4 Diapause

As mentioned above, *D. similis* and *M. calamogonus* could be feeding on different sources of food when florets of *Chionochloa* are rare. Also both those species apparently overwinter as adults, making extended diapause unlikely. However, the cecidomyiid is thought to be specific to *Chionochloa* and therefore cannot feed on alternative hosts and had to develop a different strategy to survive the low flowering years (Kelly et al., 2000; McKone et al., 2001; Rees et al., 2002).

Here I would like to discuss the general meaning of diapause and the costs and benefits it has on the insects that experience it.

Some species experience *obligatory diapause*, which is diapause of a certain length that every individual in the population experiences as a part of its life history. Other species use *facultative diapause* that is diapause, which may or may not occur in an individual or a population, and is dependent on environmental conditions established during certain key stages of the insect's development (Beck, 1968).

Tauber et al., (1986) distinguished different stages in the diapause-inducting stimuli. Later Koštál (2006) distinguished three main phases in the diapause system: pre-diapause, diapause, and post-diapause. Each phase was combined from few sub-phases (Figure 1.2). In the following summary I will use Koštál's (2006) definitions and structures supplemented by information from Tauber et al., (1986).

1.4.1 Pre-diapause stages

Environmental changes can be used as cues which the insects use prior to their diapause stage and that information is used to induce diapause. The pre-diapause stages are combined from two sub-stages: *Diapause induction* and *Preparation phase.*

Diapause induction: A genetically determined sensitive period initiates neuroendocrine, metabolic and behavioural changes, which begin to occur in the insect's systems as a result of environmental cues. Some of these aspects are similar in all species (e.g., reduced metabolism) but most of the changes are species-specific.

Preparation phase: The insects prepare for diapause. That includes build up of energy-reserves, locate a micro-habitat which will be appropriate for the diapause period, aggregation or migration.

1.4.2 Diapause stage

Arrested physiological development of the insects with alternative physiological functions, which are mostly unknown and sensitive to environmental changes. This stage is combined from three sub-stages: *Diapause initiation*, *Diapause maintenance* and *Diapause termination.*

Diapause initiation: Morphogenesis stops, metabolism is suppressed, physiological preparation occurs and the intensity of diapause rises. Mobile insects still feed to increase their reserves and search for a suitable microhabitat where the period of diapause is spent.

Diapause maintenance: Metabolic activity is very low yet constant. Duration of diapause is a species-specific characteristic and can range from several weeks to more than 10 years. Maintenance of diapause can be determined by day length, altered thermal threshold or a combination of different factors.

Diapause termination: In general, insects use stimuli they receive from external cues to trigger the termination of diapause. Such cues can be related to photoperiod, food, moisture, temperature and internal cues from insect hosts to their parasitoids and vice versa. Environmental cues shared by a population help synchronize the termination of diapause in individuals within a population. Each insect species has a different degree to which it depends on these cues to terminate diapause, with some species that cease to respond to their maintenance factors and gradually terminate their diapause.

1.4.3 Post-Diapause

After diapause termination, the organism will continue in direct development, unless the conditions are still not suitable for growth and development. In these conditions, diapause symptoms, such as suppressed development (quiescence), will persist for different periods of time.

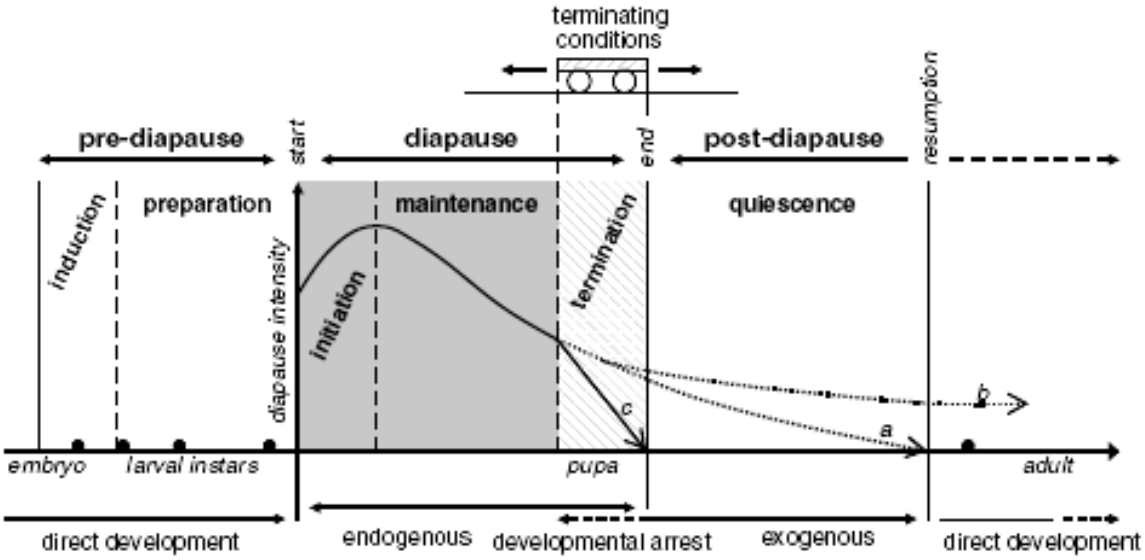

Figure 1. 2 Schematic depiction of diapause terms (see text). Thick line with arrowhead in the lower part of the picture indicates the passage of time starting from formation of zygota to the death of one hypothetical insect individual. The points on the line delineate major ontogenetic stages (different staging must be considered in different species). Three major phases, namely pre-diapause, diapause and post-diapause, are distinguished during the diapause-including ontogeny. Further division into sub-phases, namely induction, preparation, initiation, maintenance, termination and quiescence, is indicated by vertical lines (not all the phases must necessarily be found in all species and situations). Changes in diapause intensity are schematically presented: dotted branches (a and b) apply to the constant conditions, while the solid branch (c) applies to the change in environmental conditions (specific terminating conditions/stimuli coming at different physiological times - movable carriage). Detailed explanation of all terms is in the text. From Koštál (2006).

1.4.4 Types of diapause

Some insects enter diapause and emerge after one year. Others can stay in diapause for two years and longer. What controls insect emergence? What are the different strategies insects can use in order to adjust their diapause duration together with maximizing their fitness? Below are different diapause strategies I found in the literature:

1.4.4.1 Simple diapause

A simple diapause is diapause for a maximum of 12 months (overwintering) (Menu, 1993b; Menu & Debouzie, 1993; Roux et al., 1997). Individuals will emerge whether environmental conditions (e.g., food resources, weather conditions and so on), are suitable or not.

1.4.4.2 Fixed prolonged diapause

Menu, (1993b) suggested that several genotypes of diapause duration may occur within a population. Therefore the population will display variability in the duration of diapause but individuals do not display plasticity. This kind of diapause can be referred as fixed diapause, where insects in the population will emerge after a fixed number of winters but not before or

after that fixed number, and this number can vary among the different genotypes within a population.

Menu and Debouzie (1993) suggested that a fixed diapause strategy can reduce an insect's fitness because of (a) loss of reproduction opportunities, (b) mortality before emergence, (c) the probability that the year of emergence will have unsuitable conditions with no recruitment and (d) no empirical data for the advantages of such prolonged diapause. However a recent study has found empirical proof for fixed diapause. Varkonyi et al., (2002) studied periodicity in *Xestia* moths in boreal forests of the Holarctic Region, which are known to have an obscure two year cycle. They showed that the reason for this life cycle is a consequence of the host-parasite hypothesis, which suggests that univoltine specialist parasitoids cause high mortality in the host's one-year cycle cohort but lower mortality in the host two-year cycle cohort and consequently very few moths emerge after one year but many emerge after two years.

1.4.4.3 Risk-spreading diapause (Bet - hedging)

Risk-spreading diapause is a common behaviour of insects and plants that experience fluctuations in their environment. Some studies have modelled seed germination strategies in desert annual plants (Cohen, 1966; MacArthur, 1972; Venable & Lawlor, 1980) and although these models deal with plants, they can also be applied to insect diapause (Tauber et al., 1986; Hanski, 1988).

Cohen (1966) made specific predictions in his model: (1) seeds that did not germinate in suitable conditions during the first year should germinate under the same conditions in the following years (2) The proportion of germinating seeds should be positively correlated with the proportion of suitable years and (3) all genotypes should produce seeds that germinate both in the first year and in following years. The evolutionary ecology of risk-spreading diapause is explained by a trade-off between diapause and bet-hedging. A female may increase her fitness if her offspring emergence is spread over two or more years on the account of a lower proportion of offspring that emerge immediately while prolonged diapause of her offspring might be costly (e.g., predation during diapause, losses of reproduction opportunities). In uncertain fluctuating environments, the strategy that will be selected is the one that will have the highest fitness in the long term. A specific genotype may have several possible phenotypes (strategies) and insects from the same population, which are subjected to the same environmental conditions, may diapause or emerge without dependency on environmental conditions (Hopper, 1999).

Examples of risk-spreading behaviour are found in the females of the European chestnut weevil *Curculio elephas* that have 95% emergence after one or two years in diapause and the rest emerge after three or more years in diapause (Soula & Menu, 2003). Another bet-hedging genotype is found in the desert ground-nesting bee *Perdita portalis* where 73% of larvae pupated during the first summer season, 93% of the remaining larvae pupated in the second summer season and the rest stayed in diapause for a third season (Danforth, 1999).

1.4.4.4 Predictive diapause

The first to define the term 'predictive diapause' were Hanski (1989) and Roques (1989) for seed and cone insects which emerged in larger numbers from diapause in years with large cone or seed crop.

Hanski (1989); Roques (1989) and Roux and Roques (1997) suggested temperature, water stress and plant chemicals, such as gibberellins, function as biotic and abiotic factors for some insect species which may stay in diapause longer than the usual winter diapause (simple diapause) and emerge according to these cues. Predictive diapause has some recent empirical support. Diapause of the cone maggot *Strobilomyia anthracina* is negatively correlated with the number of cones available for females (Annila, 1981; Brockerhoff & Kenis, 1997). Westbrook et al., (2003) found a significant influence of temperature, relative humidity, solar radiation and precipitation on the length of diapause in the boll weevil *Anthonomus grandis* and suggested it has predictive abilities which allow it to anticipate suitable conditions for emergence. Other studies of various insects from different orders found emergence from diapause was correlated with biotic and abiotic factors in Lepidoptera (Bakke, 1963; Hedlin et al., 1982; Powell, 1989); Hymenoptera (Bakke, 1963; Claret & Carton, 1980; Brodeur & McNeil, 1989; Polgar & Hardie, 2000; Garcia et al., 2002; Maeto & Ozaki, 2003); Diptera (Bakke, 1963) and Coleoptera (Maeto & Ozaki, 2003).

1.4.5 Prolonged diapause

Prolonged diapause is diapause for twelve months or longer. The insects in extra long diapause miss one or more opportunities to reproduce, which may be used by other individuals in the population that started or ended their diapause simultaneously to the individual in extra long diapause (Hanski, 1988). Why would insects want to miss breeding opportunities? The only reason that favours extra long diapause is local adaptation to multi-annual variability among years in availability of resources (Hanski, 1988). Insects in extra long diapause attempt to increase their offspring's fitness by reproducing in a better season

with adequate resources. However, insects in diapause can be subjected to harsh environments, which can cause mortality before the chance to reproduce, and therefore diapause is costly (Hanski, 1988). Prolonged diapause is the case where insects enter diapause and stay in diapause for longer than other individuals in the population.

1.4.5.1 Prolonged diapause in hymenopteran parasitoids

Following is a short review of prolonged diapause in Hymenopteran parasitoids. The reason it is mentioned here is that hymeopteran parasitoids are not free living insects during the life stages of their prolonged diapause and therefore more complex. Diapause in Hymenopteran species is important to the parasitoid for two reasons: to maintain the population during unfavourable conditions (as with all other insects that undergo diapause) and to stay in synchrony with their hosts (Doutt et al., 1976). Parasitoids have evolved mechanisms that allow them to perceive and respond to their host's physiology (Tauber et al., 1983) in particular the host's hormonal system (Danks, 1987). For example, the calcid wasp *Melittobia chalybii* is entirely dependent on the state of the host through its diapause (Schmieder, 1933; Danks, 1987). All hymenopteran life cycle stages were observed to diapause, that is eggs inside the parental female body, all larval instars, pupae and adults of some species (Doutt et al., 1976). In addition, diapause in parasitoids shares all the features of diapause in non-parasitic insects (Tauber et al., 1986).

Parasitoids which enter diapause can do it either autonomously, or passively through their host's physiology. In many species, the parasitoid eggs hatch inside the host but the larvae arrest their development while the host continues to feed and grow (Doutt et al., 1976; Tauber et al., 1983). By this behaviour, the parasitoid gains two important things: it ensures the host is large enough for it to complete its development and synchronize its emergence with the host within and between seasons (Tauber et al., 1983). If the host enters diapause, the parasitoid will enter diapause as well and start its development just before the host instar larva moults into the pupal stage (Doutt et al., 1976; Tauber et al., 1983).

Tauber et al., (1983) suggested that the host's hormones or other internal factors of the host can act as cues for the level of diapause the host is undergoing, which the parasitoid can use to predict future conditions. When ready, the parasitoid kills the host and pupates; at this stage it is subjected to environmental conditions as any other free living insect where some species emerge immediately and others enter diapause (Doutt et al., 1976).

There are three types of interaction between insects and their parasitoids: (1) abiotic environmental cues are the main regulator of parasitoid diapause. In this situation, the

parasitoid diapause is independent to the host's diapause; (2) a combination of abiotic environmental cues and the host's physiological condition and (3) the host's physiological condition only. In this last case, the parasitoid diapause and emergence is totally dependent on the host's diapause (Tauber et al., 1983).
In general, the study of diapause is less advanced in parasitoids than in other insects (Tauber et al., 1983) perhaps because it is harder to distinguish parasitized individuals.

To summarize, diapause is known to occur in stochastic environments and different insects choose different diapause strategies to deal with stochasticity in a way that will maximize their fitness.

1.5 Sex ratios and diapause

The relationship between diapause and sex ratios has not been extensively studied. Kraaijeveld and Van Alphen (1995) studied diapause of a parasitic wasp (*Asobara tabiada*), which attack the *Drosophila* flies, *D. melanogaster* and *D. subobscura*. They found a negative relationship between sex ratio and diapause with more male-biased populations after extended diapause. They suggested that more males enter extended diapause than females. Kraaijeveld and Van Alphen (1995) also cited similar trends found in an MSc study by Adriaanse ICT (in Dutch) where the sex ratios of *Asobara tabiada* emerging after extended diapause were male skewed.

1.6 Overall aims and objectives of my study

Does mast seeding in *Chionochloa* work in terms of satiating the seed predators? Each of these three species of seed predators is affected by the unpredicted food supply of the host plant, as well as their parasitoids, which, in some species are specific to their hosts.

In this study I aimed to achieve the following goals:

1. Study the phenology and biology of the three seed predator species of *Chionochloa* tussocks, and their parasitoids in all their life stages.
2. Identify whether the three seed predators (*D. similis*, *M. calamogonus* and the undescribed cecidomyiid) and their parasitoids use prolonged diapause and, if so, which type of diapause each of the insects is using (simple, fixed, risk-spreading, or

predictive diapause).

3. If diapause in the seed predators is predictive, determine what cues the insects use to adjust their diapause.
4. Discover which are the parasitoid and hyper-parasitoid species that attack each of the insect seed predators.
5. Find out whether the parasitoids have prolonged diapause. If they do, find out whether they diapause inside or outside their hosts. Find out whether they have predictive abilities or whether they use the host's hormones to adjust their diapause. If they have predictive abilities, determine whether they use the same cues as their hosts for prediction. If not, find out which cues they use.
6. Find out whether sex ratio is influenced by diapause in any of these species.

Specifically, my hypotheses are:

1. Out of the three seed predators, the undescribed cecidomyiid is more likely to enter prolonged diapause. That is because it is the most abundant insect on *Chionochloa* plants during mast years, whereas it is comparatively less common in low-flowering years (McKone et al., 2001). Predictive abilities can be useful in helping the insect terminate diapause and therefore avoid starvation of its progeny. As a consequence it would increase its fitness and the number of predated seeds in mast years (see chapter 5). I therefore hypothesise that the undescribed cecidomyiid has predictive prolonged diapause, but not *D. similis* or *M. calamogonus*.

2. Specific parasitoids of insects that undergo prolonged diapause should be adapted to their host's biology in order to increase their fitness. I therefore hypothesise predictive abilities in the parasitoids of the undescribed cecidomyiid but not in parasitoids of *D. similis* or *M. calamogonus*.

3. Seed predators that are using prolonged diapause, adjust their life cycle to the mast seeding behaviour of their host plants by 'reading' the plant's hormone system or by using environmental cues such as temperature when diapausing away from the plant (see chapter 5). I hypothesis that the cecidomyiid will respond to similar cues as its host (*Chionochloa*) and that the parasitoid species will use similar cues as their host (the cecidomyiid).

Introduction to Chapter 2

Eucalyptodiplosis chionochloae was known mostly in its larval form for more than forty years. Colin Burrows was the first to report the existence of these larvae in his PhD thesis in 1961. He found them in the florets of several species of *Chionochloa*. Later studies reported of these 'orange larvae' but no adults were found until 1996. These adults were two females that emerged in the fridge from samples of *Chionochloa rubra* collected in Takahe Valley (Fiordland, South Island) by Dave Kelly, University of Canterbury. Later in January 2000, DK collected 18 more females ovipositing in *Chionochloa* florets from Otira Valley (central South Island). All attempts to rear these larvae to adults or locate males or pupae failed.

In October 2005 I first found females, males and pupae of this 'undescribed cecidomyiid' from plant material I collected in the previous summer season. The new findings finally allowed a formal description of the species. Our paper in the *New Zealand Journal of Zoology* provided a formal taxonomic description, presents some new biological and ecological information which was discovered during my study and describes the new methods for rearing these insects.

My contribution to this paper involved: (i) running all the technical work and insect rearing, and (ii) writing a first draft of the manuscript, excluding the taxonomic description. More specifically I wrote parts of the Introduction, Methods, and the Biology and Geographical Distribution sections, (iii) discovered the existence and location of larval prolonged diapause (iv) successfully reared males and pupae of the midges for the first time, and (v) successfully reared two specific parasitoid species, one of which is a new species that has never been reported before (see Chapter 3). Peter Kolesik wrote parts of the Methods and the taxonomic description as well as drew the images in Figures 1 and 2; Eckehard Brockerhoff and Dave Kelly wrote parts of the Introduction and the Biology and Geographical Distribution section and advised regarding the insect rearing.

2. Description of *Eucalyptodiplosis chionochloae* sp, Nov., a cecidomyiid feeding on inflorescences of *Chionochloae* (Poaceae) in New Zealand

Peter Kolesik, Michal S. Sarfati, Eckehard G. Brockerhoff and Dave Kelly, *New Zealand Journal of Zoology, 2007, Vol. 34*: 107–115

2.1 Introduction

This paper ends a search of more than 40 years for a formal description of a very widespread and important New Zealand cecidomyiid. Some insect species are discovered first as adults and formally named, and only later are aspects of their biology (e.g., host plants, life cycle) determined. In other cases, much of the biology is known years before material to complete a formal description can be obtained. This is an example of the latter case, an insect which is a key consumer of seeds in native tussock grasses, and which thus could be called the snow tussock flower midge.

New Zealand has large areas of alpine and sub alpine grassland, particularly in the South Island, dominated by grasses in the genus *Chionochloa* (Poaceae). There are 23 endemic New Zealand species of *Chionochloa* (Edgar & Connor, 2000; Connor & Lloyd, 2004), and a single species, *C. frigida*, in Australia (Mallet, 2005). This account concentrates largely on the New Zealand plants, but we return to consider the Australian species below. *Chionochloa* spp. have been much studied in New Zealand on account of their wide distribution, economic importance for extensive agriculture, and highly variable synchronous flowering ("mast seeding"). Most of the New Zealand species are 75– 150 cm tall tussock-forming ("bunchgrass") plants generally called "snow tussocks" and showing periodic flowering. At irregular intervals of 2–6 years most plants throughout the South Island flower heavily in synchrony (Kelly et al., 2000; Schauber et al., 2002). The plants produce typical wind-pollinated flower culms (inflorescences) which in *Chionochloa pallens* Zotov, as an example, are about 100–140 cm tall carrying an average of 40.5 spikelets, each made up of an average of 5.2 small florets (Kelly et al., 1992). Each floret contains three anthers and a single ovule which may ripen into a single seed. Florets of *Chionochloa* are attacked by a range of different insects, of which three are especially common (McKone et al., 2001): a moth *M. calamogonus* Meyrick (Lepidoptera: Gelechiidae), a fly *D. similis* Spencer (Diptera: Chloropidae), and the cecidomyiid which is the subject of this papers. Cecidomyiidae is a large family with 5500 described species worldwide (Gagne', 2004). With a few exceptions

the native cecidomyiids of New Zealand are not well known. The scientific name as well as the common name of the family - gall midges - relate to the habit of the larvae of many species to create abnormal growth of plant tissues known as “galls”. However, other cecidomyiids are carnivores or parasitoids, and some feed on and damage plant tissue without causing galls. Examples of the latter include the Hessian fly *Mayetiola destructor* (Say), a major pest of wheat, and the apple leaf-curling midge *Dasineura mali* Kieffer, an important pest in apple orchards, both introduced species in New Zealand (Scott, 1984).

The snow tussock flower midge was known only as larvae for 35 years. The orange larvae were first reported in 1961 by Burrows, who found them in florets from five different *Chionochloa* species across four different South Island sites. The larvae consume the developing seeds. No gall is formed and the damage to the plant is normally only visible when dissecting the florets, although if the flower heads are wet the orange colour of third instar cecidomyiid larvae can be evident in the field (McKone et al., 2001). Following Burrows, several subsequent papers extended the geographic and host plant range of these unidentified cecidomyiid larvae (Mark, 1965c; Burrows, 1968; McKone et al., 2001), which was eventually extended to include every species of *Chionochloa* which has been examined (*Chionochloa australis*, *C. conspicua*, *C. crassiuscula*, *C. flavescens*, *C. macra*, *C. oreophila*, *C. pallens*, *C. rigida*, *C. rubra*, *C. spiralis* and *C. teretifolia* - for plant authorities see (Edgar & Connor, 2000)), and almost every examined site, from the central North Island (39°S) to Stewart Island (47°S; see map in (McKone et al., 2001)). In the absence of adults, larval DNA Inter Simple Sequence Repeats (ISSRs) were used to test for cecidomyiid speciation by host plant or geographic location, and all tested larvae appeared to belong to a single species (McKone et al., 2001; McCall et al., 2004).

The snow tussock flower midge is not only wide spread, but also damages many *Chionochloa* florets. A major survey of floret damage throughout the central South Island (White, 1975) showed that *Chionochloa* florets suffered heavy losses to herbivores, but most of the damage was attributed to *M. calamogonus* and *D. similis*, with few cecidomyiids seen. Subsequent work showed that this was probably partly due to sampling early in the flowering season, as the cecidomyiid is most abundant towards the end of the season (McKone et al., 2001). Studies at one site from 1986–95 (Mt Hutt, Canterbury) found overall losses of florets to all predators averaged 58% (Kelly & Sullivan, 1997), including losses of 44 and 57% in 2 years (1986 and 1990, respectively) when most of the insects seen were cecidomyiids (Kelly et al.,

1992). On individual plants the snow tussock flower midge can destroy more than 60% of florets (Figure 5 in McKone et al., (McKone et al., 2001).

This cecidomyiid has also been recognised as important because of its apparent role in driving mast seeding (highly variable synchronous flowering among years) by its host *Chionochloa* plants. All *Chionochloa* species for which data exist show mast seeding, and these species show some of the most extreme variation across years of any plants worldwide (Kelly et al., 2000). Various possible factors can select for masting in a plant species (Kelly, 1994), but in *Chionochloa* it has been shown that the primary benefit is predator satiation (Kelly & Sullivan, 1997; Sullivan & Kelly, 2000; Kelly et al., 2001). Mast seeding is selectively advantageous to *Chionochloa* because the insect seed predators are satiated during the occasional high-flowering years and starved in low- and non-flowering years, reducing losses of florets from c. 70% in low-flowering years to c. 10% in high-flowering years (Kelly & Sullivan, 1997). However, life cycle features of the snow tussock flower midge were thought to make it much harder to satiate than *M. calamogonus* or *D. similis* (McKone et al., 2001), and thus the extreme mast seeding found in *Chionochloa* is thought to be primarily driven by selective pressures from the cecidomyiid (Kelly et al., 2000; Rees et al., 2002). Hence, there has been particular interest in this cecidomyiid for many years.

Unfortunately, for such a common and wide spread insect, life cycle stages other than larvae proved difficult to find. The first adults seen (two females) were reared at the University of Canterbury in 1996 from *Chionochloa rubra* material collected in Takahe Valley (Fiordland). One of these females was illustrated in McKone et al., (2001), who also reported the collection of 18 more females found ovipositing in the field in the upper Otira valley, central South Island, on 13 January 2000. However, systematic attempts to rear adults or locate adult males or pupae had proved fruitless (McKone et al., 2001). Here, we report successfully rearing pupae and adults, allowing at last a full description. The species is formally described and named here, and placed in *Eucalyptodiplosis*, a genus previously containing two species feeding on leaf and branch buds of *Eucalyptus* trees in Australia.

2.2 Material and Methods

To obtain cecidomyiid eggs and larvae, inflorescences of *Chionochloa pallens* and *C. macra* were collected at Mt Hutt, New Zealand, at 1070 m altitude (43°32.04′S, 171°32.97′E) and at 1300 m altitude (43°31.15′S, 171°32.61′E). The 1070 m site is the same location used in previous studies (Kelly et al., 1992; Rees et al., 2002). Collections were made every 5 days

from early December 2004 to mid March 2005. Florets were dissected under a stereomicroscope to find eggs and larvae of *E. chionochloae*; these were preserved in 70% ethanol. In addition, a bulk collection of inflorescences of *C. pallens* was made in mid February 2005. These inflorescences were placed outside in the shade, among leaf litter under trees at the University of Canterbury campus (20 m altitude) over winter (March–October 2005) to allow immature insects to develop. The samples were contained in nylon 1 mm mesh bags to provide ambient moisture conditions, wrapped inside 5 mm wire mesh to exclude mammalian predators. In October, the inflorescences were taken to the lab and placed inside a chamber to collect emerging adults. Emergence started a few days later, and male and female *E. chionochloae* were collected, sexed and preserved as mentioned above. After emergence ceased in mid December, 30 random inflorescences were dissected to search for any insects still in the larval stage to determine whether such individuals are in prolonged diapause. Plant material from Mt Hutt containing cecidomyiid larvae was also placed overwinter on site at Mt Hutt using similar mesh bags placed at the base of tussocks. Emergence of adults at this field site was recorded using emergence chambers checked weekly from late October 2005 to March 2006.

Canada balsam mounts of larvae, pupa and adults were prepared according to the technique outlined in Kolesik et al., (2005). Length measurements were made with a microscope imaging system. Wing length corresponds to distance between the base of C vein and the wing apex, wing width to the maximum width perpendicular to C vein. Measurements refer to the type series. Drawings were made with the aid of a camera lucida. Terminology of adults and pupae follows Gagné (1981), larvae follow Gagné (1989). The types and other material are deposited in the New Zealand Arthopod Collection, Auckland (NZAC) and the South Australian Museum, Adelaide (SAMA).

2.3 Description

2.3.1 Genus *Eucalyptodiplosis* Kolesik 2002

(Kolesik et al., 2002) in Australian Journal of Entomology 41, p. 24.

2.3.1.1 Type Species

E. germinis, by original designation *Eucalyptodiplosis* is a genus hitherto containing two species that induce leaf and branch bud galls on *eucalypts* in Australia. *Eucalyptodiplosis germinis* Kolesik feeds on *Eucalyptus cosmophylla* F. Muell. and *Eucalyptus camaldulenis* Dehnh., and *Eucalyptodiplosis mcintyrei* Kolesik feeds on *Eucalyptus grandis* Hill ex Maiden

(Kolesik et al., 2002). The genus belongs to the tribe Cecidomyiini (sensu (Gagné, 1994)) and is characterised by the toothed tarsal claws that are bent beyond mid length, the presence of an occipital protuberance on the head, male flagellomeres bearing three circumfilar whorls, an aedeagus that is long and blunt, and a protrusible ovipositor with long fleshy cerci.

2.3.2 *Eucalyptodiplosis chionochloae* Kolesik sp. nov.

2.3.2.1 Type Material:

Holotype male, New Zealand, Mt Hutt (43°32.04′S, 171°32.97′E, 1070 m a.s.l.), emerged 24 Dec 2005, ex florets of *Chionochloa pallens* Zotov, collected 18 Feb 2005 by Michal Sarfati, (NZAC). Paratypes: 2 males, 3 females, 1 pupa, 3 larvae (NZAC, 2 males, 2 females, 3 larvae (SAMA, I21797–I21803), same data as holotype.

2.3.2.2 Other Material

5 larvae, New Zealand, Mt Hutt (43°31.15′S, 171°32.61′E, 1300 m a.s.l.), ex florets of *Chionochloa macra* Zotov, collected 15 Feb 2005 by Michal Sarfati (NZAC).

2.3.2.3 DNA Analysis

ISSRs analysed for larvae from *C. pallens*, *C. rubra* Zotov, *C. crassiuscula* (Kirk) Zotov and *C. oreophila* (Petrie) Zotov collected at Mt Hutt (43°32′S, 171°32′E), Gertrude Saddle (44°44′S, 168°01′E), Lewis Pass (42°23′S, 172°23′E) and Temple Basin (42°55′S, 171°35′E) (McCall et al., 2004).

2.3.3 Description

Male (Figures 2.1a–f, 2.4a). Colour. Eyes black, scape and pedicel grey, flagellomeres and head pale brown, thorax pale brown with three dorsal, longitudinal, dark-brown stripes, abdominal sclerites brown, unsclerotised parts orange, legs grey-brown. Head. Antennae: scape as wide as long; pedicel slightly wider than long, narrowed distally; flagellomeres 12 in number, not fused, binodal, with one whorl of looped circumfila on proximal and two on distal nodes, circumfilar loops short, not reaching next distal whorl, nodes setulose, neck asetulose. Eye facets round, eye bridge 2–3 facets long. Postvertical protuberance wider than long, bearing a pair of setae apically. Palpus 4-segmented, segments progressively longer, segment 2 widest. Frons with 3–4 setae per side. Thorax. Wing 2.42 mm (range 2.34–2.50, n = 5) long, 0.81 mm (0.78–0.86) wide, RS in form of spur, situated half way between arculus and end of R1; R5 slightly bent at its juncture with RS, joining C at wing apex. Tarsal claws toothed, curved beyond mid length, tooth narrow, empodia slightly longer than claws. Abdomen. Sclerites entire, first narrow, remaining trapezoid, with pair of anterior trichoidal papillae. Setae: on sternite 1 in posterior line, on sternites 2–8 in posterior line and mesal

band, that is narrow in anterior segments and wide in posterior segments where covering nearly entire sclerites; on tergites 1–6 in posterior line and small group mesolaterally, tergite 7 with small group mesolaterally, tergite 8 asetose except anterior pair of trichoidal papillae. Genitalia: gonocoxite elongate, cylindrical, gonocoxal apodemes merged; gonostyle gradually tapered and bowed towards aedeagus, setulose on basal fourth dorsally and ventrally and third posteriorly, ridged beyond; aedeagus stout, substantially longer than cerci and hypoproct, evenly wide at distal third, obtuse apically, with several asetose papillae on distal half; hypoproct bilobed, with deep incision between lobes, each lobe with two setae apically; cerci triangular, with long setae.

Female (Figures 2.2a–d, 2.4b). Head. Antennae: flagellomeres 12 in number, cylindrical, with simple circumfila. Postvertical protuberance less prominent than in male. Thorax. Wing 2.68 mm (2.50–2.80, n = 5) long, 0.94 mm (0.86–1.02) wide. Abdomen. Sternites 1–7 rectangular, tergites 1–8 trapezoid. Position of setae: sternites and tergites 1–7 as in male, tergite 8 with anterior pair of trichoidal papillae and a few setae posteriorly. Genitalia: ovipositor protrusible, long; cerci large, fleshy, 5 times longer than wide, setose, with a few thick sensory setae apically; hypoproct short, trapezoid in dorso-ventral view, narrow laterally, with pair of setae apically. Colour and other characters as in male.

Pupa (Figures 2.2e, f, 2.4c).Colour. Antennal horns, prothoracic spiracle and dorsal spines dark brown, remaining parts pale brown. Length 2.54 mm. Antennal horns angular, short, wide. Facial papillae not recognisable on available specimen due to insufficient clearing during preparation. Prothoracic spiracle long, with trachea ending at its apex. Abdominal segments 2–8 dorsally with fields of simple spines on anterior half, number of spines similar on segments 2–7, smaller on segment 8.

Larva (Figures 2.2g–j, 2.3b). Colour orange. Length 2.01 mm (1.92–2.12, n = 6). Integument with spiculae. Head with posterolateral apodemes half length of head capsule. Sternal spatula with long shaft and bilobed apical enlargement, anteriorly flanked by single sternal, two triplets of lateral and single ventral papillae, all asetose. Terminal segment with 4 papillae on either side, from outer to inner: medium sized corniform, medium sized with short stout seta, large corniform, small with short stout seta.

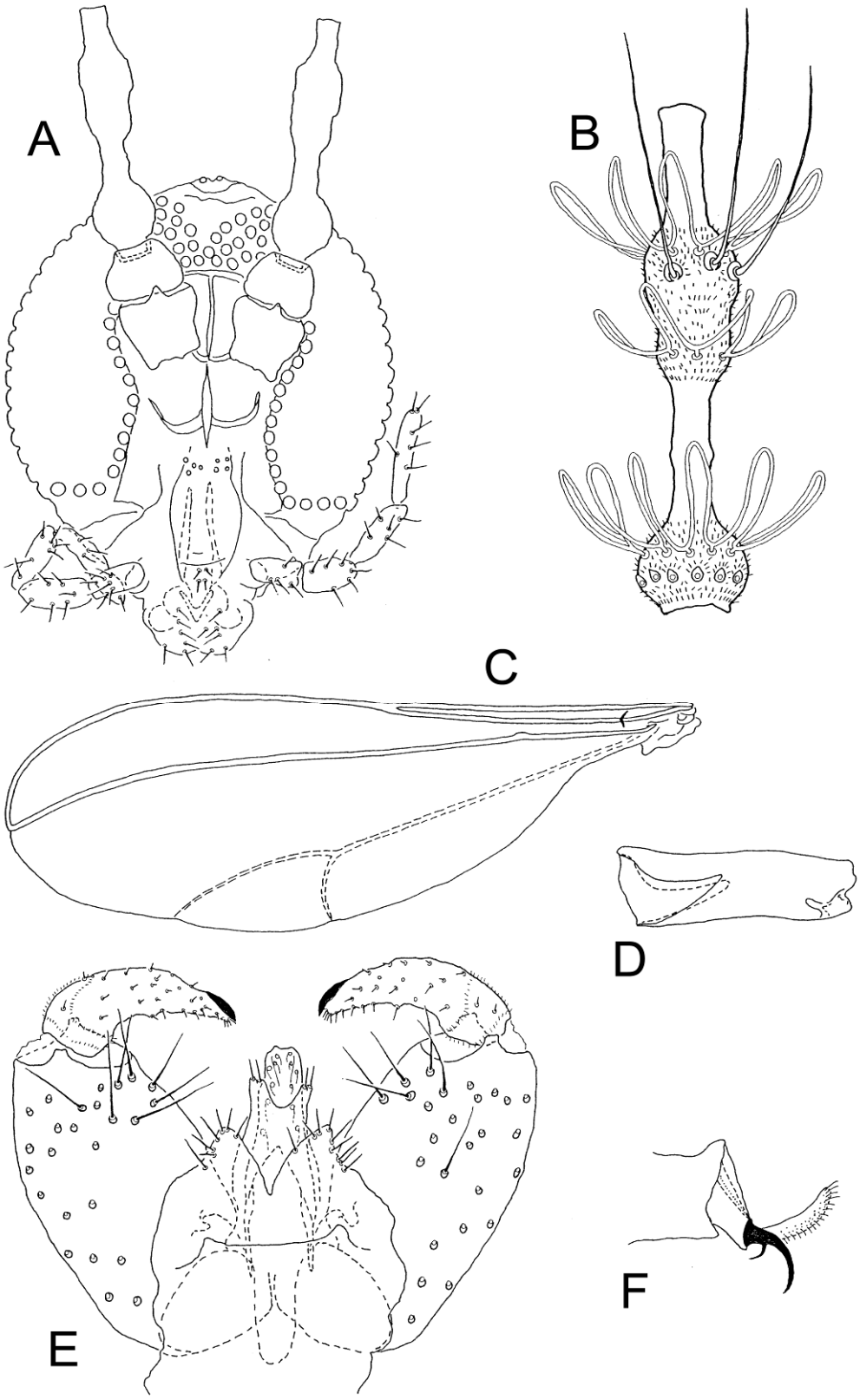

Figure 2. 1 Male *Eucalyptodiplosis chionochloae*. A, Head frontally; B, flagellomere 6; C, wing; D, first tarsomere; E, genitalia dorsally; F, tarsal claw with empodium.

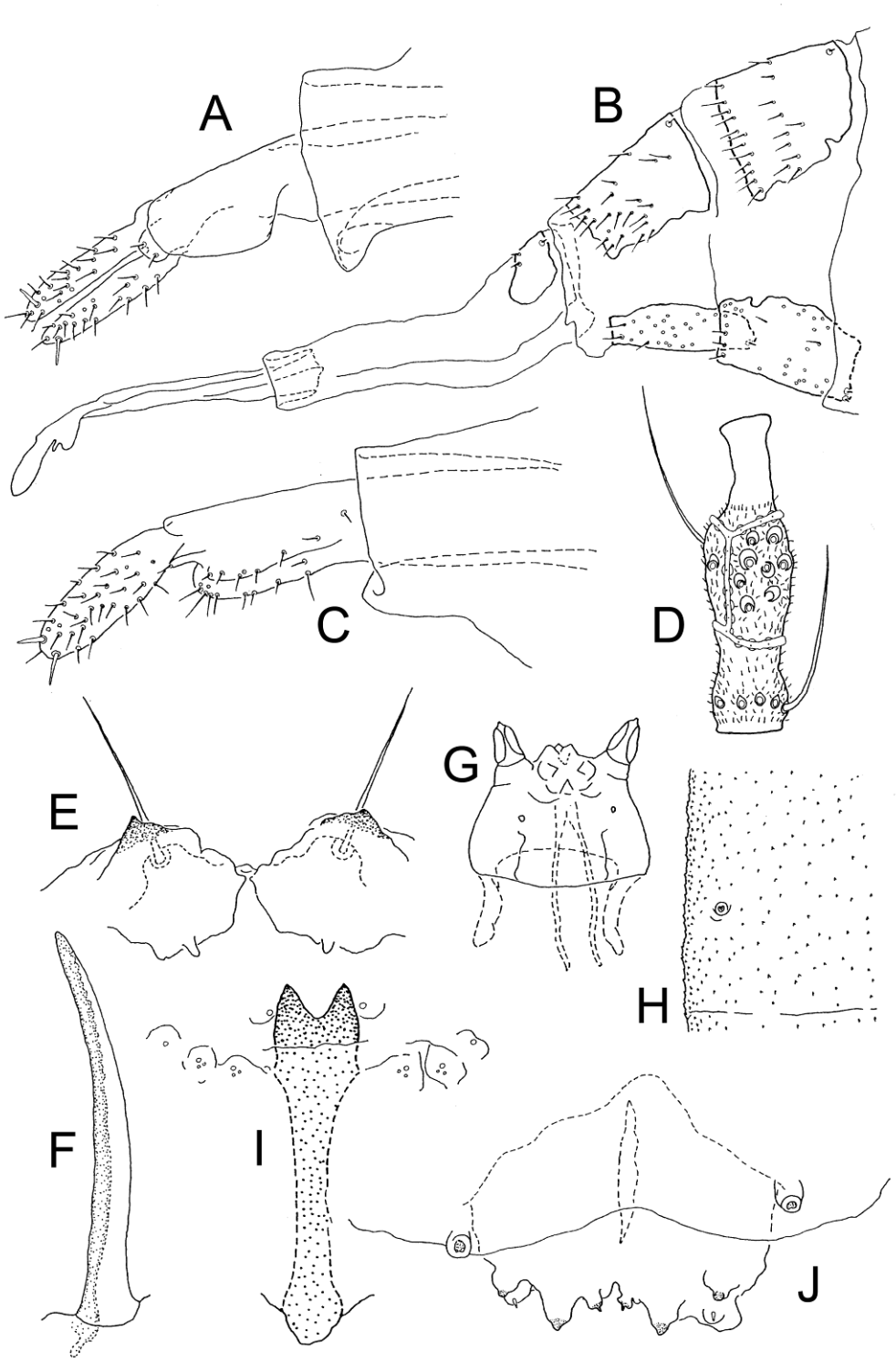

Figure 2. 2 *Eucalyptodiplosis chionochloae*. A–D, Female; E, F, pupa; G–J, larva. (A), End of ovipositor with cerci and hypoproct ventrally; (B), end of abdomen laterally; (C), end of ovipositor laterally; (D), flagellomere 6; (E), antennal horns and cephalic papillae; (F), prothoracic spiracle; (G), head ventrally; (H), integument with spiracle; (I) sternal spatula with adjacent papillae; (J) terminal segment dorsally.

2.3.3.1 Etymology

The specific name of the new species is derived from the generic name of the host plants.

2.3.3.2 Biology and Geographical Distribution

E. chionochloae do not induce galls, unlike the two Australian species in the genus *Eucalyptodiplosis* (Kolesik et al., 2002). Instead, the larvae feed externally on immature, developing seeds of various *Chionochloa* species (McKone et al., 2001). Adult females have been observed ovipositing into florets at about the time of anthesis in the Otira valley (42°53.8′S, 171°32.6′E, 1000 m altitude) on 13 January 2000. At the Mt Hutt 1070 m site in the 1995/96 season, the number of eggs found peaked on 2 January and no eggs were found after 16 January (McKone et al., 2001) which also corresponded with anthesis at that site. In the 2004/05 season at the 1070 m site, eggs were found from early January to late February. The eggs hatch rapidly into first instar larvae. In agreement with McKone et al., (2001), we recognised three larval instars (last instar is distinguished by the presence of sternal spatula- see Figure 2.2i), with older instars being less mobile; all instars stay within the floret, close to the seed or attached to it (Figure 2.3). Larval feeding prevents formation of the seed. Illustrations of early and late instar larvae are given in McKone et al., (2001). At the 1070 m Mt Hutt site, larvae were found in 1996 between 2 January and the last sample on 16 February, and in 2005 between early January and early March. In both cases, third instar larvae were still common in florets in the last sample of the season. Contrary to McKone et al., (2001) it is now known that larvae do not drop from the florets to diapause in the soil. Instead, larvae remain within the florets which separate from the inflorescence and fall to the ground late in the season (late February – early April) where the larvae diapause without creating a larval cocoon (Figure 2.3b).

The pupal stage lasted only 2–3 days in the lab, consistent with the fact that pupae have been rarely observed. Emergence of adults in the field at Mt Hutt began on 7 December 2005 on the 1070 m site and continued until 19 January 2006. Adults emerging from the bulk collection kept over winter outside at the University of Canterbury campus had a slightly female-biased sex ratio (56% female). Adults appear to be capable flyers in the lab and the field, unlike the other *Chionochloa* flower-feeding fly *D. similis* (Chloropidae), whose adults in the lab fly only reluctantly and for short distances (Kelly et al., 1992).

Eucalyptodiplosis chionochloae is univoltine; however, it has been previously suggested that some larvae can enter extended diapause instead of emerging after one winter (Kelly et al., 2000; McKone et al., 2001). The existence of extended diapause was confirmed in two ways. First, examination of the bulk collections placed over winter at the University of Canterbury after first-year emergence of adults had ceased in December 2005, revealed at least 23 live

third-instar larvae remaining inside florets in the plant material. Second, all the material was then placed outdoors for a second winter, and during October and early November 2006, 122 adult male and female insects emerged from this collection. Extended diapause is known in other cecidomyiids that specialise in feeding on developing seeds or inflorescences, such as *Contarinia oregonensis* Foote which infests seed cones of Douglas fir (Hedlin, 1964). Larvae of the wheat midge, *Sitodiplosis mosellana* (Géhin), are known to have spent up to 12 winters in extended diapause (Barnes, 1952), although the majority of their larvae tend to spend only one winter in diapause (Wise & Lamb, 2004).

In the emergence cages we found adults of two hymenopteran parasitoid species that are likely to be associated with *E. chionochloae*, *Gastrancistrus* sp. (Pteromalidae: Pireninae) and an unknown Platygastrinae (Platygastridae) (Jo Berry pers. comm.). Both these parasitoid wasps were also collected while ovipositing into *Chionochloa* florets in the Otira valley on 13 January 2000, coinciding with *E. chionochloae* oviposition. It is not yet known whether the two wasps oviposit into *E. chionochloae* eggs and/or larvae.

As described by McKone et al., (2001) and outlined in the Introduction, the known host-plant range of *E. chionochloae* includes all 11 species of *Chionochloa* which have been searched for the insect in New Zealand. The currently known geographical range is essentially every site where careful examination has been carried out on late-season *Chionochloa* florets, from the central North Island to Stewart Island (see map in McKone et al., ((2001)). It has yet to be ascertained whether *E. chionochloae* or a related cecidomyiid species is found in Australia on *C. frigida* (the only *Chionochloa* species found in Australia, which is the native range of the other two species in *Eucalyptodiplosis*).

Figure 2. 3 *Chionochloa pallens* florets. A, Healthy seed; B, infested floret with diapausing third instar larva of *Eucalyptodiplosis chionochloae*.

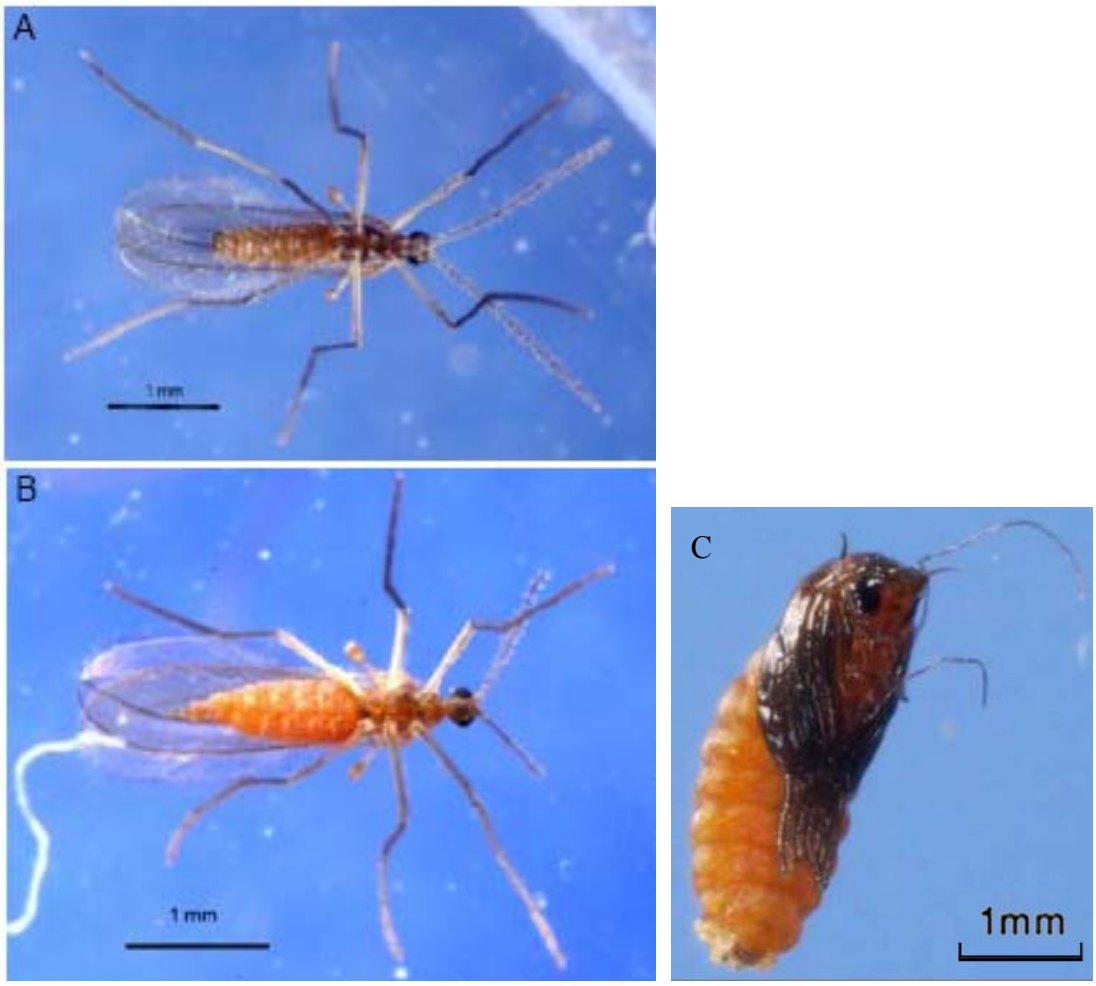

Figure 2. 4 Ventral views of adults of *Eucalyptodiplosis chionochloae*. A, Male; B, female and C, ventral/lateral view of a pupa.

2.3.3.3 Remarks

Morphologically, the new gall midge fits *Eucalyptodiplosis* (a genus comprising two Australian species that feed on *Eucalyptus* (Myrtaceae)) in all characters except for the presence of tergite 8 in females of *E. chionochloae*. In the light of the level of morphological similarity among the three known species in this group, we conveniently place the new gall midge in this genus. The fact that the host range of *Eucalyptodiplosis* now includes plants from such distantly related families as Myrtaceae and Poaceae is not unusual in Cecidomyiidae. These two plant families belong to the respective host range of *Contarinia*, *Dasineura* and *Lasioptera*, large genera that radiated to feed on a vast number of host plant families (see Gagné (2004)). *Eucalyptodiplosis chionochloae* differs morphologically from its two Australian congeners in the following characters: in adults the first and second flagellomeres are separated; in the male the aedeagus does not reach the posterior edge of the gonocoxites, the gonostyle is tapered and bowed gradually, and the circumfilar whorl on the anterior flagellomeral node reaches the mid length of the flagellomeral neck; in the female the abdominal segment 8 bears a tergite; in the larva the anterior lobes of the spatula are acute,

and the outermost papilla on the terminal segment is corniform. In contrast, *E. germinis* and *E. mcintyrei* have the first and second flagellomeres in adults fused; in the male the aedeagus reaches beyond the posterior edge of the gonocoxites, the gonostyle is tapered and bowed at the distal fourth, and the circumfilar whorl on the anterior flagellomeral node does not reach the mid length of the flagellomeral neck; in the female abdominal segment 8 bears no tergite; in the larvae the anterior lobes of the spatula are round, and the out-most papilla on the terminal segment bears a short seta.

Introduction to Chapter 3

A new species of an unidentified platygastrid parasitoid wasp of *Eucalyptodiplosis chionochloae* was first discovered in October 2005, together with the first discovery of its host's adult males and pupae. These platygastrid wasps have never been reported before despite substantial study of the host species. Although most *E. chionochloae* larvae were previously collected later in the season after larvae were already parasitized, infested larvae have never been identified before and signs of infestation were never reported in the literature. *E. chionochloae* were never successfully reared to adults (see Chapter 2) and neither were adults of its two species of parasitoids. Male and female *Gastrancistrus* sp. (Hymenoptera: Pteromalidae) were previously observed by Jon Sullivan and Angela Cone around florets containing *E. chionochloae* and therefore were suspected to be associated with *E. chionochloae*. However, most sampling of *E. chionochloae* was done later in the season, which correlates with the time *Gastrancistrus* adults are mating and ovipositing. Although three males of the unidentified platygastrid wasp were collected in Otira Valley by Dave Kelly in January 2000 (together with one female of *Gastrancistrus* sp. and 18 females of *E. chionochloae*), these wasps were never sent for identification and only five years later, Jo Berry (Landcare Research, Auckland) identified them for me.

After searching for some time for a taxonomist specializing in the Platygastridae family, I located Peter Buhl from Denmark (University of Copenhagen) and he identified these wasps as undescribed species from the genus *Zelostemma.* He also agreed to formally describe the species and collaborate with writing a multidisciplinary paper combining taxonomy, ecology and biology. Our paper in the *New Zealand Journal of Zoology* contains a formal taxonomic description, some new biological and ecological information which was discovered during this time of study and methods for rearing these insects.

Another issue relevant to this paper, which was not discussed in the publication, is the early emergence of *Z. chionochloae* before its host *E. chionochloae*. Below are several hypotheses I came up with that may explain this early emergence:

1. The parasitoids may emerge before their host but mate only later in the season, when eggs and first instar larvae of their host are available.
2. *Z. chionochloae* may partition its resources with *Gastrancistrus* sp. (another specific parasitoid to *E. chionochloae*) to avoid interspecific competition, and may infect the eggs, (which are present earlier) rather than the first instar larvae.

3. *Z. chionochloae* may compete with *Gastrancistrus* sp. and gain benefits from infecting the host first (see discussion in Chapter 5 for possible interactions between *Z. chionochloae* and *Gastrancistrus* sp.).
4. *Z. chionochloae* may not be specific to *E. chionochloae* and feed earlier in the season on another host. This hypothesis however seems less likely as the complex life cycle of prolonged diapause which can take more than three years and which matches the prolonged diapause of *E. chionochloae*, was probably selected in order for *Z. chionochloae* to better exploit a host which has variable and unexpected abundance. Prolonged diapause in *Z. chionochloae* would probably be less strongly selected for if it could have exploited another host.

My contribution to this paper involved: (i) running all the technical work and insect rearing, (ii) writing a first draft of the whole manuscript, excluding the taxonomic description, more specifically writing a significant part of the Introduction, Methods, Data Analysis and Biology sections; (iii) running data analysis, calculating the results and constructing graphs, and (iii) discovering the existence of larval prolonged diapause inside the host. Peter Buhl wrote the Material Examined, the Diagnosis, Description, Affinities and Etymology sections, and took the photos for Figures 1 to 11 using SEM (Scanning Electron Microscope); Eckehard Brockerhoff and Dave Kelly wrote parts of the Introduction, Data Analysis, and Biology sections and advised me regarding the host's rearing.

3. Description, phenology and biology of *Zelostemma chionochloae* Buhl sp. nov., a platygastrid wasp feeding on *Eucalyptodiplosis chionochloae* (Diptera: Cecidomyiidae) in New Zealand

Peter Neerup Buhl, Michal S. Sarfati, Eckehard G. Brockerhoff *and* Dave Kelly (2008) *NZ Journal of Zoology* 35:255-264.

3.1 Introduction

The cecidomyiid midge *Eucalyptodiplosis chionochloae* Kolesik was known for over 40 years in its larval and egg stages, but it was only recently formally described (Kolesik et al., 2007) following the first rearing of adult males and females. *Eucalyptodiplosis chionochloae*, the snow tussock flower midge, is a seed feeder on *Chionochloa* tussock grasses which exhibit mast seeding (extreme variation of flowering among years; (Kelly et al., 2000)). In addition, our studies of this cecidomyiid also revealed two undescribed parasitic wasp species attacking *E. chionochloae*: a species of *Gastrancistrus* (Pteromalidae: Pireninae) (Cone 1995; J. Berry, pers. comm.) and a newly discovered species in the genus *Zelostemma* (Platygastridae: Platygastrinae) (see Masner & Huggert (1989)). There are currently several other known species in the genus *Zelostemma*: *Z. oleariae* (Maskell), a parasitoid of a gall midge attacking *Olearia forsteri* (now *O. paniculata*), and an undescribed species that parasitises a gall midge on *Senecio* (now *Brachyglottis*) *stewartiae* (Masner & Huggert 1989).

Zelostemma chionochloae is a natural enemy of a specialised seed predator of *Chionochloa* grasses. As a host-specific seed predator, local populations of *E. chionochloae* would not be able to survive zero-flowering years because of a lack of breeding sites for the larvae. However, our recent studies have shown that this cecidomyiid can enter prolonged diapause to escape non-flowering years (Kolesik et al., 2007). Similarly, parasitic wasps are dependent on their hosts for the development of the wasp larvae. Thus, among competing lineages of parasitoids of diapausing insects, those with the greatest selective advantage will be the ones that adapt their development, including their diapause behaviour, to that of their hosts. Many other seed-feeding insects and their parasitoids undergo prolonged diapause (e.g. Annila 1981; Brockerhoff & Kenis 1997; Wise & Lamb 2004), and there is often a synchrony in development between hosts and parasitoids, as the parasitoids have to adapt to their host's developmental and physiological conditions (Tauber et al., 1986). *Zelostemma chionochloae* is likely to play an important indirect role in the mast seeding ecology of these grasses by

regulating the seed predator populations that are key factors affecting the reproductive success of *Chionochloa* species.

In this paper, we give a description of *Zelostemma chionochloae*, an endemic species that parasitises the snow tussock flower midge *E. chionochloae* in New Zealand. We also provide information on its biology, ecology and phenology. We demonstrate that *Z. chionochloae* can enter prolonged diapause, and show how its emergence is related to that of its host. *Zelostemma chionochloae*, which belongs to the Platygastridae family, is now added to a list of 1322 valid species described worldwide with 69 recognized genera (Norm Johnson, per. comm.; Hymenoptera On Line Database (2007), see references).

3.2 Material and Methods

3.2.1 Study sites

The two study sites at Mt Hutt, Canterbury, New Zealand were at 450 m altitude (43° 33.93' S, 171° 33.26' E) and 1070 m altitude (43° 32.05' S, 171° 33.03' E). The 1070 m site is dominated by *Chionochloa pallens* Zotov, and is the same location used in previous studies (Kelly et al., 1992; Rees et al., 2002; Kolesik et al., 2007). The 450 m site contains large *C. rubra* Zotov that grow on a southeast-facing slope amongst otherwise exotic grassland vegetation. This site is a commercial farm paddock and is exposed to sheep grazing a few times a year.

3.2.2 Sampling methods

Randomly selected inflorescences from 10 *Chionochloa rubra* and 7 *Chionochloa pallens* plants were counted and collected during mid-February 2005, from the 450 m and 1070 m sites respectively. Inflorescences of each plant were kept separate and sealed inside 1 mm nylon-mesh bags with a small amount of soil. These bags were placed in unglazed clay pots and left in the field, buried at the study sites with their rims level with the soil surface. This rearing method ensured that the insects diapausing inside the florets were exposed to near-natural conditions of temperature, humidity and photoperiod, and also enabled the collection of the insects later in the spring.

During mid-October 2005, each mesh bag was opened and covered with an emergence trap made from a white plastic pot, with two transparent 5 ml vials attached open to the inside, so that emerging insects (*E. chionochloae, Z. chionochloae* and *Gastrancistrus* sp.) could move into the transparent vials. Insects that emerged at each of the two sites from mid-October 2005 to early March 2006 were collected weekly, counted, identified to species, sexed and preserved in 70% ethanol. A few insects that could not be reliably sexed were excluded from the analysis of sex ratios (see below). After emergence stopped, the plastic pots were removed and mesh bags were sealed again. The inflorescences were left in the field for another winter, and the same collection procedure was used again from October 2006 to March 2007. Emergence in the 2006/07 summer season represents insects with prolonged diapause, i.e. those insects which remained in diapause during the 2005/06 summer season.

A similar collection of flowering material was made in February 2006 at the 450 m site and kept in the field till first year emergence was recorded in the 2006/07 summer.

3.2.3 Data Analysis

The sex ratio of *Z. chionochloae* was tested against a 50:50 expectation with χ^2 tests. Parasitism rates were calculated as the percentage of total adult *Z. chionochloae* divided by the total number of adult insects found (*E. chionochloae*, *Z. chionochloae* and *Gastrancistrus* sp.).

***Zelostemma chionochloae* Buhl sp. nov.**

3.2.4 Material Examined

Holotype female: New Zealand, Mt Hutt, 450 m, reared 13.xii.2006, from cecidomyiid on *Chionochloa rubra*, coll. ii.06 by M. Sarfati. Preserved in the Auckland Museum, New Zealand, AMNZ80715. Paratypes: 59 females, 7 males same data as holotype. Preserved in the Zoological Museum, University of Copenhagen and Auckland Museum (including 2 males, AMNZ80716). Preserved in 70% ethanol.

3.2.4 Diagnosis

A less than 2 mm long, dark-legged species with preapical antennal segment of female 1.3 times as long as wide; A10 of male more than twice as long as A9.

3.3 Description

Female: Body length 1.5-1.8 mm. Black; extreme apex of all femora, and base and apex of fore tibia dark brownish.

Here Figs 1-4

Head from above (Fig. 1) 1.8 times as wide as long, very slightly wider than mesosoma across tegulae, weakly reticulate-coriaceous; frons smooth in more than medial half, from anterior ocellus to the few weak transverse striae above antennal insertions. OOL:LOL = 3:4 where OOL = distance between lateral ocellus and eye and LOL = distance between lateral and anterior ocelli; longer diameter of lateral ocellus two-thirds as long as OOL. Head in frontal view 1.3 times as wide as high. Antenna (Fig. 2) with A1 fully as long as height of head, 1.3 times as long as distance between inner orbits; A3 1.2 times as long as A2, 3.5 times as long as wide, twice as long as A4; A9 1.3 times as long as wide; A10 1.3 times as long as A9.

Mesosoma (Fig. 3) 1.5 times as long as wide, as wide as high. Sides of pronotum densely hairy all over, finely reticulate-coriaceous, in lower half smooth. Mesoscutum (Fig. 4) densely and evenly hairy, finely and uniformly dull reticulate-coriaceous; notauli complete, deep and smooth; mid-lobe not prolonged posteriorly; grooves between mesoscutum and scutellum moderately wide, with a few long hairs. Mesopleuron smooth, with a few wrinkles along upper margin. Scutellum (Fig. 4) sculptured and hairy as mesoscutum. Metapleuron with dense pilosity all over. Propodeal carinae inconspicuous, area between them about as long as wide, with uneven surface.

Here Figs 5-11

Forewing (Figs 5-6) barely 0.9 times as long as entire body, overreaching tip of metasoma by a distance equal to combined length of T3-T6, 2.4 times as long as wide, with brownish tint; microtrichia dense and moderately long; submarginal vein distinct to about one-fourth the length of wing but without knob, veins M + Cu and RS + m distinctly indicated as nebulous traces, RS less clearly; marginal cilia 0.08 times as long as width of wing. Hindwing (Fig. 7) 5.8 times as long as wide, with two hamuli; marginal cilia one-third the width of wing.

Metasoma (Fig. 8) 1.1-1.3 times as long as head and mesosoma combined, as wide as mesosoma. T1 slightly swollen, densely longitudinal striated, medially smooth. T2 at anterior margin with eight very short longitudinal furrows, about 0.08 as long as tergite, and with two large, pubescent basal foveae behind them, T2 otherwise smooth. T3-T6 with faint traces of reticulation over most of surface and with many scattered, superficially implanted long hairs.

Male: Body length 1.3-1.7 mm. Antenna (Fig. 9) with A3-A4 of equal length and width, A4 (Fig. 10) only slightly modified; A5-A8 each about one and a third times as long as wide; A9 as long as wide; A10 2.3 times as long as A9; flagellar pubescence one-third as long as width of segments. Metasoma (Fig. 11) as long as head and mesosoma combined.

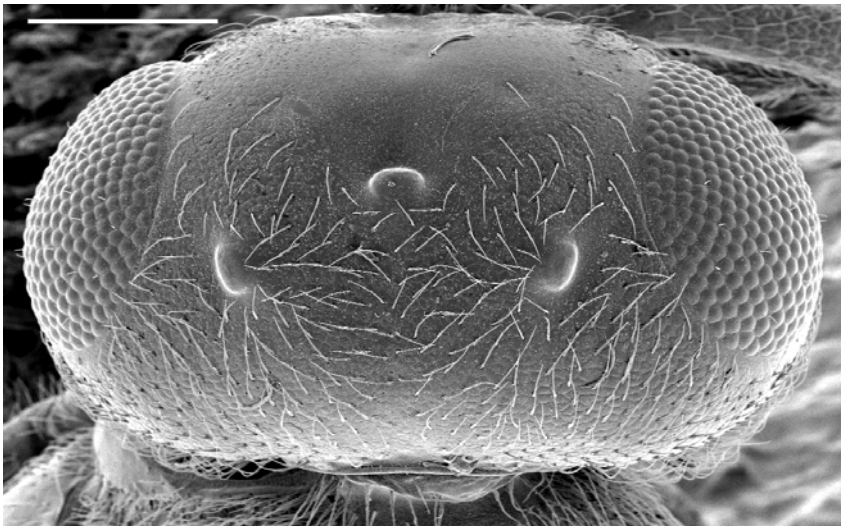

Figure 3. 1 *Zelostemma chionochloae* Head from above. Scale bars = 100 μm

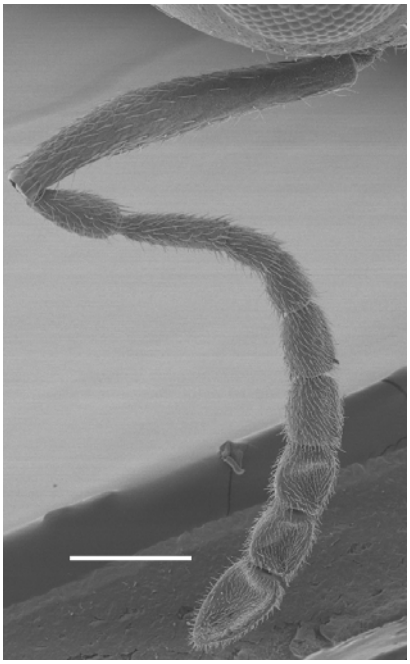

Figure 3. 2 Female antenna. Scale bars = 100 μm

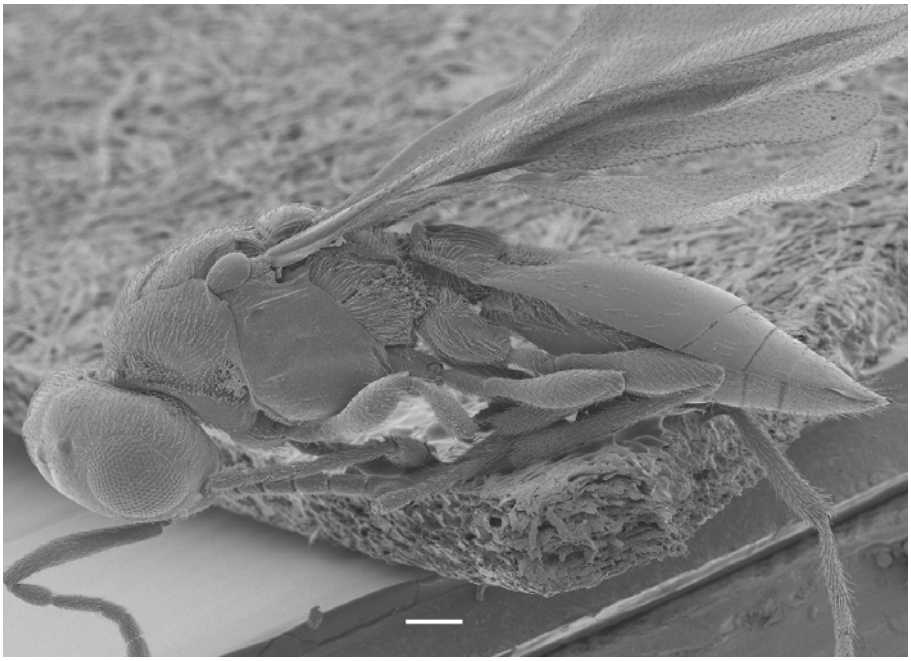

Figure 3. 3 Female in lateral view. Scale bars = 100 μm

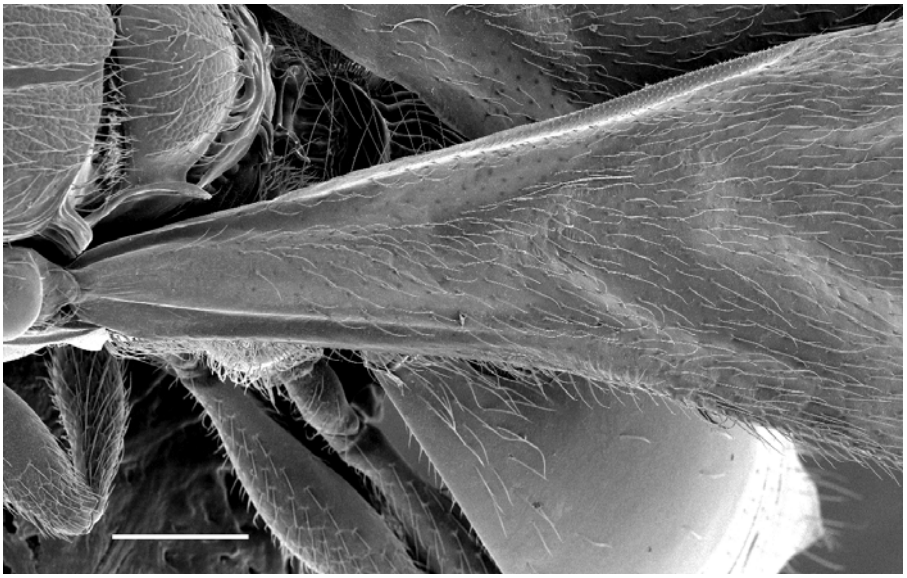

Figure 3. 4 Mesoscutum and scutellum from above. Scale bars = 100 μm

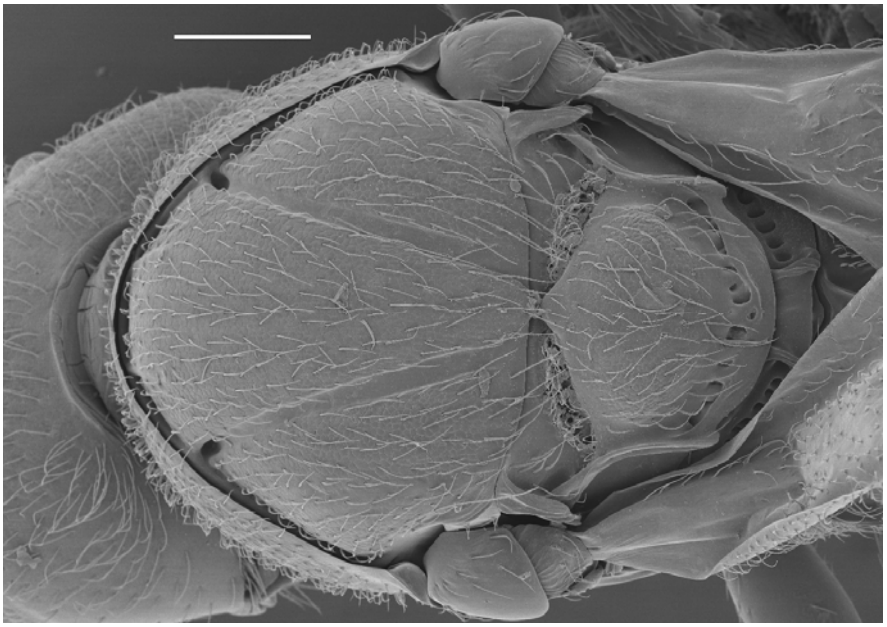

Figure 3. 5 Base of forewing. Scale bars = 100 μm

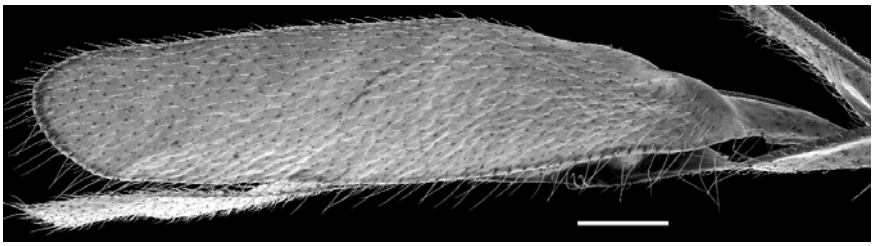

Figure 3. 6 Apex of forewing. Scale bars = 100 μm

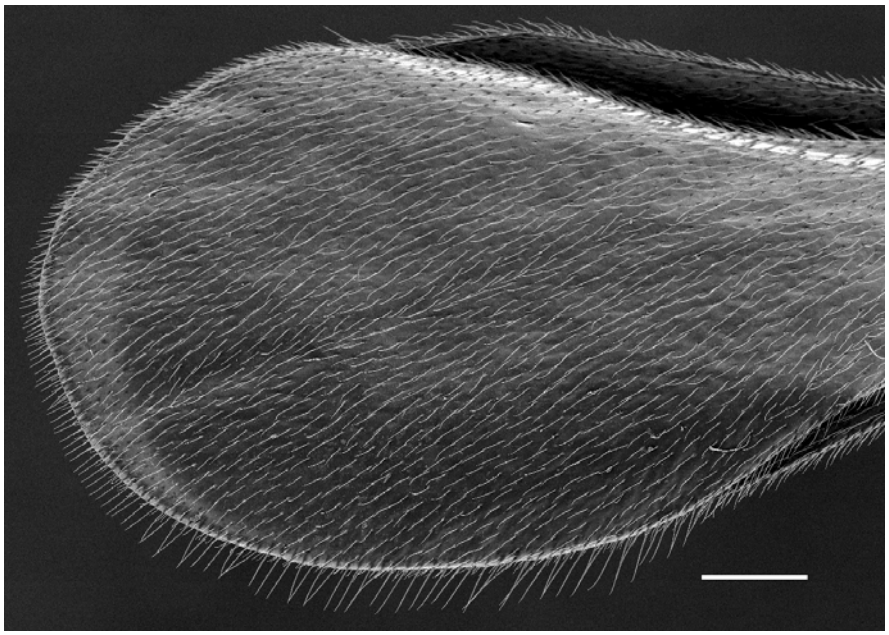

Figure 3. 7 Hindwing. Scale bars = 100 μm

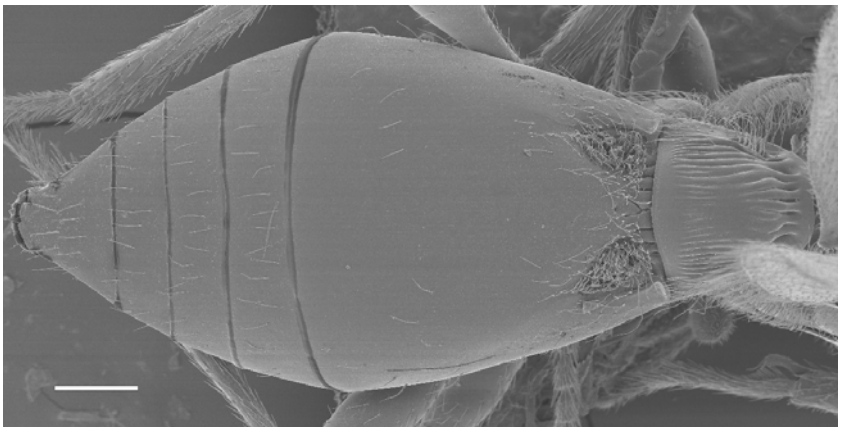

Figure 3. 8 Female metasoma from above. Scale bars = 100 μm

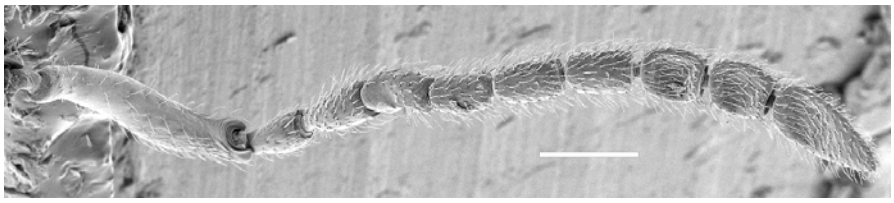

Figure 3. 9 Male antenna. Scale bars = 100 μm

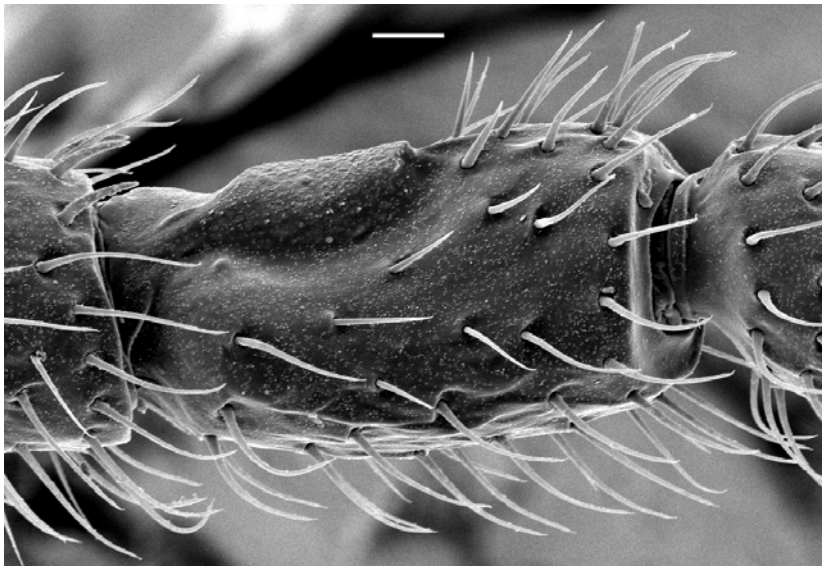

Figure 3. 10 Male antennal segment 4. Scale bars = 10 μm

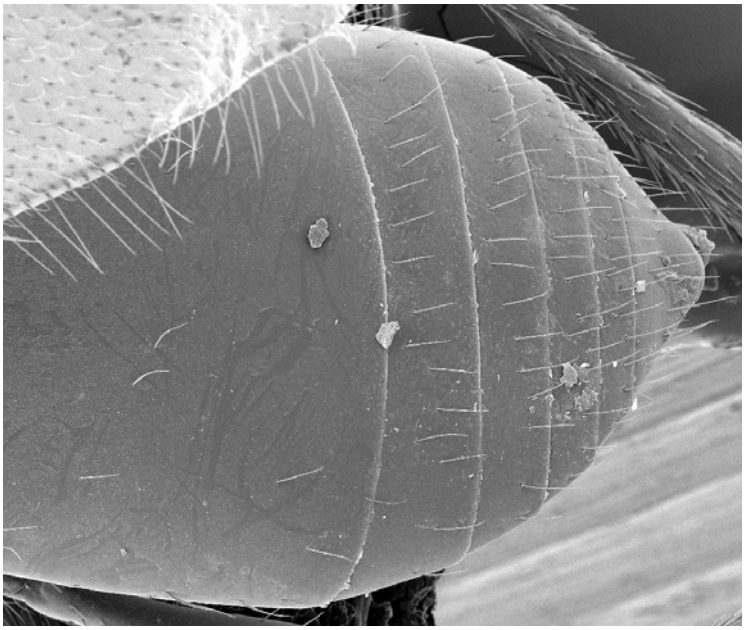

Figure 3. 11 Apex of male metasoma. Scale bars = 100 μm

3.4 Affinities

This species differs from the only other described species of the genus, *Z. oleariae* (Maskell 1888) in having more slender female antennae (especially A3 and preapical segments) and relatively longer male A10 (which is less than twice as long as A9 in *Z. oleariae*). *Z. oleariae* also has more transverse head and lighter body appendages than *Z. chionochloae*, and it is larger (2.3 mm). Cf. Masner & Huggert (1989). Material was also compared with a determined female specimen (homotype) of *Z. oleariae* preserved in the Natural History Museum, London.

3.5 Etymology

The specific name of the new species is derived from the specific name of the cecidomyiid host.

3.6 Biology

Zelostemma chionochloae emerged from the February 2005 collections in much higher numbers at the 450 m site (206 adults in total over both years) than at the 1070 m altitude site (5 adults in total over both years). The emergence of *Z. chionochloae* began about 1-2 weeks before that of its host, *E. chionochloae*, but was generally well synchronized with it (Fig. 12).

This ensured the presence in the field of the host's eggs and first instar larvae which are probably the stage attacked by the female parasitoid (See Chapter 5). In both years, males emerged from diapause before females (protandry) which gives them the best chance to mate with more females (Figure 13). Adult males were previously observed close to ovipositing *E. chionochloae* females in Otira valley (42° 53.8'S, 171° 32.6'E, 1000 m altitude) on 13 January 2000 (McKone et al., 2001 and D. Kelly. unpublished data). According to Austin et al., (2005), most platygastrid species attack either the egg or the first instar larva of their cecidomyiid hosts. We do not know whether *Z. chionochloae* females infest their host's eggs, early instars or both, but development is completed in the late instar larval stage. *Eucalyptodiplosis chionochloae* are univoltine and some individuals enter extended diapause (Kolesik et al., 2007) and the same is true of *Z. chionochloae.* In our collection at the 450 m site, nearly as many insects (83) emerged in the second summer after extended diapause as had emerged in the first summer (123).

The geographical range of *Z. chionochloae* is unknown, but because it appears to be host specific to *E. chionochloae*, it probably has a similar geographic distribution as its host, which has been found from the central North Island, including Mt Taranaki, to Stewart Island (McKone et al., 2001; Kolesik et al., 2007). However, *Z. chionochloae* may prefer lower elevations because during our study much higher numbers emerged at 450 m altitude than at 1070 m altitude.

Parasitism rates differed between years and also between sites. At 450 m altitude, parasitism levels were 35% and 14% *Z. chionochloae* for all insects emerging during the 2005/06 and 2006/07 summer seasons, respectively. At 1070 m altitude, parasitism levels were 3% and 6.3% during 2005/06 and 2006/07, respectively.

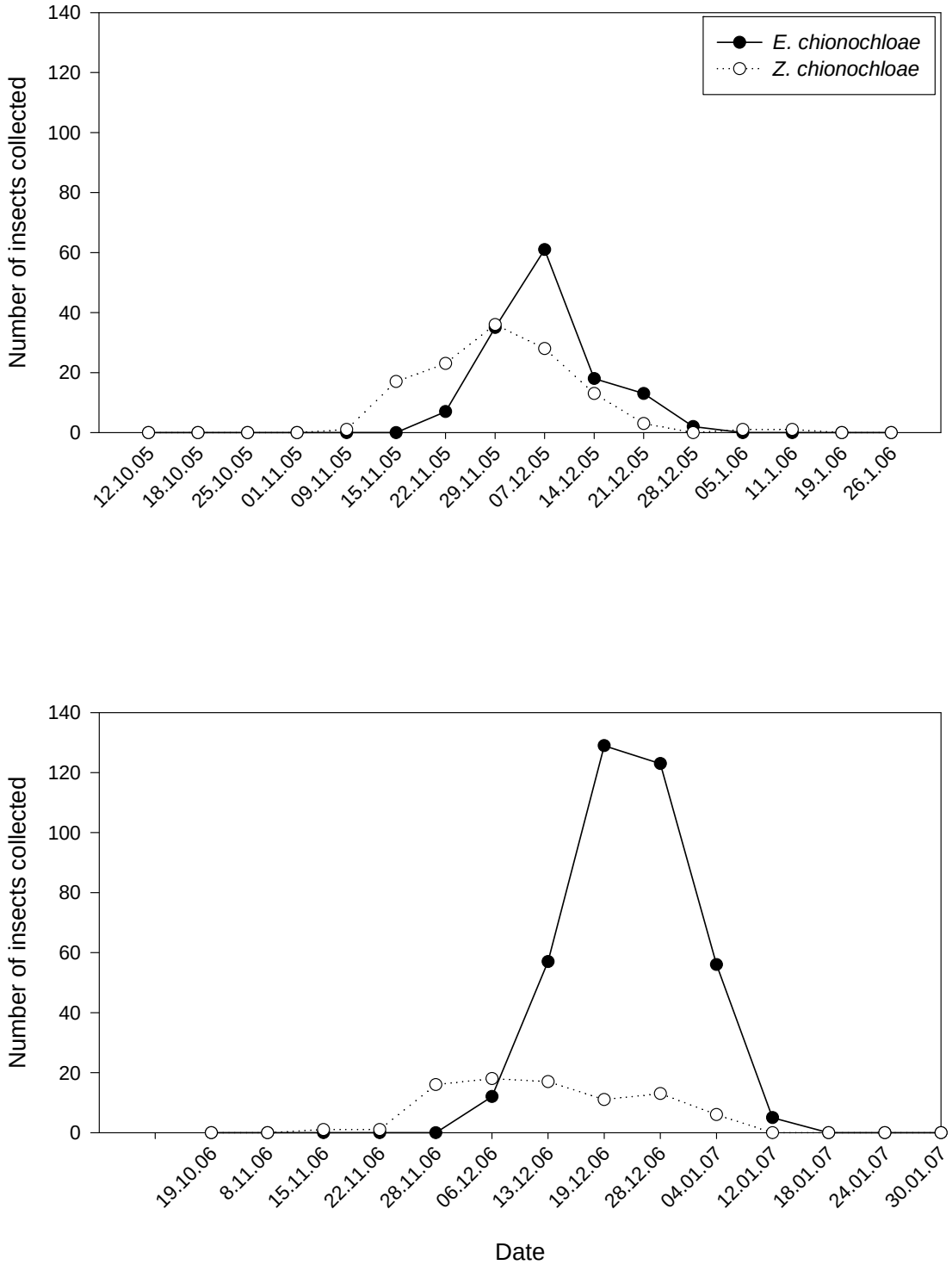

Figure 3. 12 Phenology of adult emergence of *Zelostemma chionochloae* and its host *Eucalyptodiplosis chionochloae* in (a) 2005/06 and (b) 2006/07 summer seasons at the 450 m site, Mt Hutt.

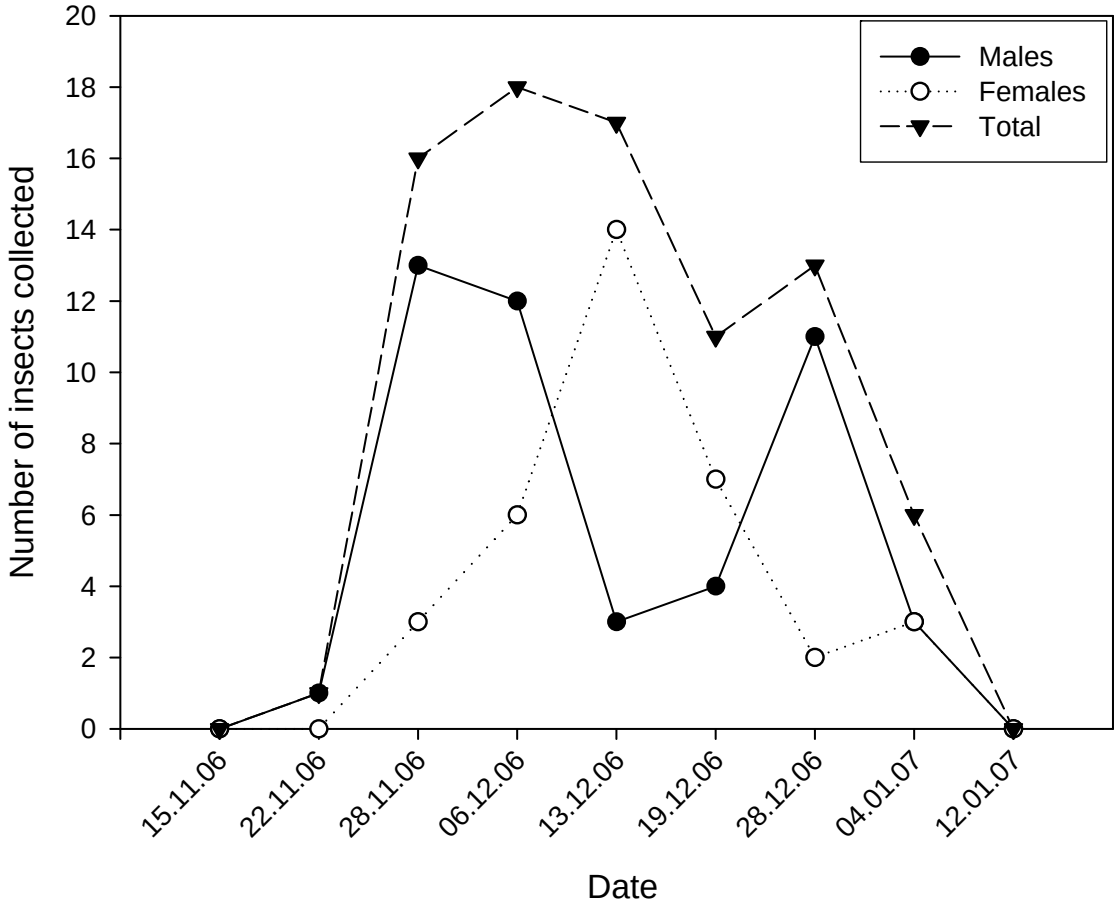

Figure 3. 13 Male and female *Zelostemma chionochloae* emergence from 450 m 2005 collection in 2006/07 summer (after two years of prolonged diapause).

The sex ratio at 450 m altitude was significantly female-biased among insects emerging during 2005/06 (n = 104, 63.5% females, $\chi^2 = 3.769$, df = 1, $P = 0.006$) but non-significantly male-biased in the second year of emergence (n = 82, 43% females, $\chi^2 = 1.756$, df = 1, $P = 0.185$). This appeared to be variation among years rather than variation due to length of diapause, because the first-year emergence in 2006/07 of the 2006 collection also had a slight male bias (n = 198, 43% females, $\chi^2 = 3.414$, df = 1, $P = 0.065$), very similar to the 2005 collection emerging in that 2006/07 season.

Other hymenopteran species have male-biased populations due to diapause: Kraaijeveld & Van Alphen (1995) stated that more males entered diapause than females in the *Drosophila* parasitoid *Asobara tabida* (Hymenoptera: Braconidae). Christiansen-Weniger & Hardie (1999) found male-biased population in the aphid parasitoid *Aphidius ervi* (Hymenoptera: Aphidiidae) reared on diapausing hosts compared with non-diapausing hosts. Christiansen-Weniger & Hardie (1999) suggested that females under certain conditions of photoperiod or

diapause may die in their larval stages without killing the host, and therefore shift the sex ratio to a male-biased population. Indeed Ellers & Van Alphen (2002) studied the fitness of male and female *Asobara tabida* during diapause stages and found that diapause reduced dry weight, energy reserves and egg load in females. It was concluded that diapause is more costly to female than male parasitoids.

Koinobiont monophagous endoparasitoids such as *Z. chionochloae*, which have a delayed development inside their living hosts, may possibly respond to the changes in the hosts' hormone levels that are associated with diapause (Beckage 1985; Lawrence 1986; Lawrence 1990). Although our knowledge of the biology of most platygastrids is patchy, some species are known to enter prolonged diapause inside their cecidomyiid hosts (Speyer & Waede 1956; Bakke 1963; Sunose 1978). Parasitic wasps can experience the same problems in finding prey as their hosts in finding food, therefore the ways in which these two groups of insects can exploit their resources may be similar (Smith & Balda 1979). A similar foraging behaviour and a set of clues and adaptations used by two species of insects where one preys the other will result in a control of one population by the other (Smith & Balda 1979).

Eucalyptodiplosis chionochloae is thought to have an important role in the flowering ecology of its host plants, *Chionochloa* spp. *Chionochloa* species have some of the most extreme mast seeding of any species worldwide (Kelly et al., 2000), and it was shown that predator satiation is the key factor to explain mast seeding for this genus (Kelly & Sullivan 1997; Sullivan & Kelly 2000; Kelly et al., 2001). *Eucalyptodiplosis chionochloae* is thought to be the main seed predator driving *Chionochloa* species to mast seeding as it is harder to satiate than the other two seed/flower predators *Diplotoxa similis*, a chloropid fly and *Megacraspedus calamogonus*, a gelechiid moth (Kelly et al., 2000; McKone et al., 2001). McKone et al., (2001) illustrated the relative abundances across years of *E. chionochloae* and *D. similis*: in low flowering years, there are more *D. similis* per floret than *E. chionochloae*, because *E. chionochloae* are apparently more likely to stay in prolonged diapause in low-flowering years. However, in high-flowering years *E. chionochloae* significantly increase in numbers, and the ratio of *E. chionochloae* per *D. similis* increases greatly. The net effect is that the abundances of *E. chionochloae* are at least as variable as abundances of *Chionochloa* flowers, perhaps even more so. The fluctuations in host population shows the importance of extended diapause to *Z. chionochloae*, which would probably be at a selective advantage if it could synchronize with its host by using the same environmental and plant cues to control diapause. In addition,

Z. chionochloae probably suffers inter-specific competition with the other *E. chionochloae* parasitoid species, *Gastrancistrus* sp. These two parasitoids are using the same resources and are experiencing the same problems of fluctuations in food supply. There may therefore be an advantage for *Z. chionochloae* to have a diapause regime that differs from that of *Gastrancistrus* sp. What regulates the diapause of *Z. chionochloae* (and *Gastrancistrus* sp. and *Eucalyptodiplosis chionochloae*), and whether parasitoid diapause termination is affected by the host's hormones or by other environmental cues, is yet to be studied.

4. Phenology, predation levels and inter-specific competition of *Chionochloa* pre-dispersal seed predators

4.1 Introduction

There are three main known invertebrates that occur on *Chionochloa* flowers and seeds: a chloropid fly (*D. similis* Spencer 1977), a gelechiid moth (*M. calamogonus* Meyrick 1885) and the recently described cecidomyiid fly *E. chionochloae* (Chapter 2 andMcKone et al., 2001). In addition, unidentified thrips (Thysanoptera: Terebrantia: Thripidae: Thripinae) and unidentified mealy bugs (Hemiptera: Pseudococcidae) are found in *Chionochloa* florets and under leaf sheath and blades (Cone, 1995). These two latter species may or may not feed on the florets or seeds but there is not enough information to estimate their damage (Sullivan, 1993; Cone, 1995). However, there is clear evidence that the three main species cause extensive damage to *Chionochloa* seeds or flowers (White, 1975; Kelly et al., 1992; Kelly & Sullivan, 1997; Kelly et al., 2000; McKone et al., 2001). Therefore I will not discuss the thrips and pseudococcid in this study any further and will focus on the three main seed/flower predators.

D. similis and *M. calamogonus* are both univoltine and overwinter as adults (McKone et al., 2001), whereas *E. chionochloae* can have a univoltine life cycle or enter extended larval diapause for at least two years (Kolesik et al., 2007) (hereafter referred to as 'prolonged diapause').

Schoener (1974) and Masters and Brown (1997) suggested that there are three possible interactions for resource partitioning of phytophagous insects that feed on the same host plant: (a) Spatial separation – spatially separated herbivore insects that share the same host plant at the same time but feed in different niches; (b) Temporal separation – herbivore insects, that share the same niches and the same host plant but are separated in time; and (c) Spatio-temporal separation – herbivore insects, which are temporarily separated in space and feed at different times, and either share the same niches and guilds or not. It has been suggested that not only direct effects of competition (i.e., reduced quantity of resources

available to competitors) can reduce competitor performance (i.e., fecundity, oviposition preference, abundance, growth rate, body size, developmental time and survival), but also indirect effects of competing species may change insect performance (Kaplan & Denno, 2007). Such indirect effects were found in many studies and related to reduced plant fitness (Harrison & Karban, 1986; Agrawal, 1999, , 2000; Wise & Weinberg, 2002; Van Zandt & Agrawal, 2004a, 2004b; Viswanathan et al., 2005).

These three seed predator insects co-occur in the same micro-habitat and feed on the same source of food. However, each species feeds on a different part or developmental stage of the floret, and the appearance of larvae of each insect is synchronized with the required developmental stage of the floret (Sullivan & Kelly, 2000). Despite this partial temporal separation, there is potentially significant interspecific and intraspecific competition for oviposition sites among these species, particularly in non-mast years when resources are scarce (McKone et al., 2001). This constraint probably affects *D. similis* and *M. calamogonus* more than *E. chionochloae* which can escape in time by entering prolonged diapause, although on the other hand *E. chionochloae* is thought to feed later within the season which would put it at a competitive disadvantage compared to *D. similis* and *M. calamogonus*. These interspecific relationships are made more complex by the presence of several parasitoids that were previously poorly studied (see Chapter 5). Recently, Kelly et al., (2008) found strong evidence for predator satiation in a 20 year dataset of *Chionochloa* studied at Mt. Hutt. In short, they found that low flowering years usually result in high predation levels (>70% of florets), whereas in high flowering years total predation by the three insects is significantly reduced to often below 10%. Although predation levels (as percentage of florets) are usually high in low flowering years (Kelly & Sullivan, 1997; Kelly et al., 2008), the actual number of florets which are available for the seed predators is smaller than in high flowering years. Therefore, in low flowering years, interspecific competition is expected to be much harsher.

Because *Chionochloa* species occur over a wide altitudinal range, there is also a possibility that seed predators may partition resources in space (elevation). As one would expect, there is a temporal response in the plants, with cooler temperatures at higher altitudes leading to these plants flowering later in the season than plants at lower elevations (Mark, 1965a; McKone et al., 1998). However, less is known about the phenology of the seed predators, and their

distribution across different elevations (McKone et al., 2001), although the abundance of the seed predators generally decreases with elevation (Sullivan & Kelly, 2000; Hay et al., 2008). The phenology and distribution of other insect species are known to be influenced by altitudinal differences in temperature (e.g., the pine processionary moth (Battisti et al., 2005)).

Here I report the results of a study on the *Chionochloa* seed predators on three different *Chionochloa* species at three different sites (each species is dominant one of these sites which are located in different altitudes) over three years to address the following questions:

1. Do predation levels by the insects follow the predator satiation theory, which suggests that percentages of predation will decrease with increasing flowering intensity?
2. How does the abundance of the different *Chionochloa*-feeding insects vary among different sites (host plants and altitude) and years? Do the insects partition their resources in time and space?
3. Which of the insects emerge first and does the phenology of these insects follow an altitudinal (temperature) gradient?

4.2 Methods

4.2.1 Study site

The study area is located on Mount Hutt in Canterbury, New Zealand, on the eastern edge of the central Southern Alps, approximately 110 km west of Christchurch. Three different sites at three different elevations were studied:

450 m site: located at the bottom of the mountain (43° 33.93' S, 171° 33.26' E), with *Chionochloa rubra* surrounded by exotic grasses. This site is a privately owned farm paddock and is exposed to sheep grazing a few times a year (Figure 4.1).

1070 m site: located half way up the skifield road (43°32.04´S, 171°32.97´E) dominated by 94% *C. pallens* and 6% *C. macra* (Kelly & Sullivan, 1997) (Figure 4.1).

1300 m site: at 43°31.15´S, 171°32.61´E with *C. macra* in addition to other native plants, such as *Aciphylla aurea* and *Celmisia spectabilis*. This site is facing southeast and is highly exposed to wind (Figure 4.1).

The 1070 m and 1300 m sites are on reserve land administered by the New Zealand Department of Conservation. These two sites were previously described in other papers (McKone, 1990; Kelly & Sullivan, 1997; Kolesik et al., 2007; Hay et al., 2008) while the 450 m site is described in Chapter 3 (Buhl et al., 2008).

4.2.2 Flowering intensity measurement

Following Kelly et al., (1992), and Kelly et al., (2000), *Chionochloa* flowering intensity was measured by counting the number of inflorescences per tussock of 60 individual tussocks from the 450 m and 1070 m sites and 30 individual tussocks from the 1300 m site. The plants were tagged and recounted every year in early February. No inflorescence counts were made at 1300 m in the 2005/06 year so flowering effort there was estimated based on the mean change over the two previous years from a higher and a lower site on Mt. Hutt. From the 1070 m site the mean change was calculated as the mean inflorescences of 2005/06 divided by the mean inflorescences of 2004/05, which gave a mean increase of 8.61-fold (Table 4.1) and D. Kelly's permanent plots in *C. macra* at 1540 m (unpublished data) used a similar calculation which gave a 4.54-fold increase. An average of these two values (6.58-fold increase, see Table 4.1) was then multiplied by the 2004/05 mean inflorescences per tussock at the 1300 m site (2.82 x 6.58 = 18.54). Therefore I estimated the mean inflorescences per tussock in the 2005/06 flowering season in the 1300 m site as 18.54.

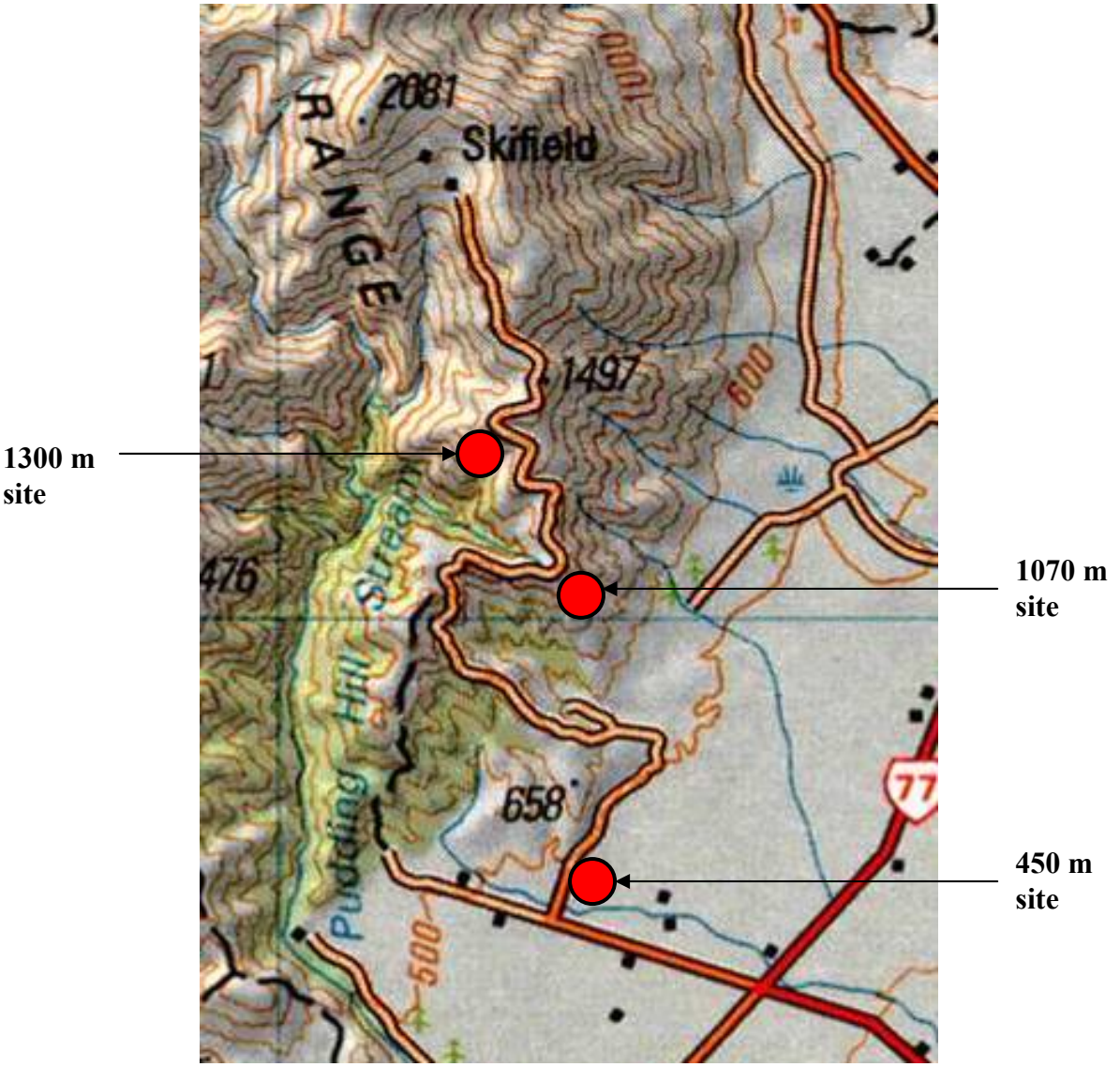

Figure 4. 1 Topographic map of the 450 m, 1070 m and 1300 m sites.

4.2.3 Sampling of immature stages of seed predators

To study the phenology of immature stages and the abundance of each insect throughout the different elevations, dissections of florets were carried out during three flowering seasons. During the flowering season of 2004/05, fifteen inflorescences were collected (from 15 different *Chionochloa* plants) every 5 days from mid-December 2004 to mid-March 2005 from each of the three altitudes. Inflorescences from the 450 m site were not collected regularly at the beginning of that season so there are some missing data from that site. Thirty spikelets per inflorescence were dissected under a stereo microscope in order to find eggs, larvae or pupae of the seed predator insects. The presence of insects, their life cycle stage, the

collection date, site and the species of host plant were noted in addition to the number of spikelets in each inflorescence and the number of florets in each spikelet. Over 56,400 florets were dissected during this flowering season across the three elevations.

During the 2005/06 and 2006/07 summer seasons, fifteen inflorescences were collected (one per plant) from each of the sites only once late in the season (early February from the 450 m site and late February from the 1070 m and 1300 m sites), after the insects had damaged the florets. This did not allow estimation of the timing of different life cycle stages within the later seasons, but did allow an estimate of the total full-season level of damage by each insect (see McKone et al., 2001). The type of damage to the florets was noted according to the different damage each species of insect causes (White, 1975; Kelly et al., 1992; Sullivan, 1993; Cone, 1995; Sullivan & Kelly, 2000). In short: there were five general categories: unpredated healthy floret, live insect present in the floret, insect remains in floret (e.g., hatched eggs, empty puparia, shed caterpillar head capsule), signs of insect damage in the floret (e.g. frass, stains on lemma and palea), empty floret. Each insect leaves traces and signs of its presence which are quite distinctive due to each insect's characteristic feeding behaviour. For example, *M. calamogonus* feed on the undeveloped ovary and palea, relatively early in the season and leave holes, partly chewed ovaries and pellety faecal material. *D. similis* feed on the anthers and leave them with a characteristic matted appearance. *E. chionochloae* feed later in the season on the developing seed and leave a flat or wrinkled seed. Around 2,300 florets were dissected in the 2005/06 flowering season from all three sites and 2052 were dissected in the 2006/07 flowering season. These observations allow an estimation of the percentage of florets that had contained each of the three insect species, which can be used as an index of insect abundance between different flowering seasons with different flowering intensities in each of the three years.

4.2.4 Phenology of adults

Collections of adults were done in two ways:

4.2.4.1 Large emergence traps

During the summer season of 2004/05, 15 emergence traps were placed over individual *Chionochloa pallens* plants at 1070 m and nine traps over *C. macra* plants at 1300 m

elevation. In the 2005/06 summer season, five traps were placed over other individual plants at each of the 450 m (*C. rubra*) and 1070 m (*C. pallens*) sites. Traps were pyramid-shaped and consisted of a wooden frame made from 2.5 cm × 2.5 cm timber stakes over which white 1 mm-mesh sheer polyester fabric was placed from ground level to the peak of the pyramid such that an entire plant could be enclosed without insects being able to escape. At the peak of the pyramid, an inverted clear plastic jar 'funnelled' insects into a smaller plastic jar containing 70% ethanol to collect and preserve all insects that moved from the tussocks up towards the light. Emergence traps were checked and jars with ethanol replaced at 5-7 day intervals from early December until late March in the 2004/05 summer season and from early November to early March in the 2005/06 summer season. To my knowledge, this spanned the emergence period of all the insects of interest. Insects collected in the jars were sorted and the *Chionochloa* seed predator insects as well as their parasitoids found in the jars were kept separately, identified and counted. The prevalence of each species was calculated in relation to the altitude and time of year.

4.2.4.2 Small emergence traps

4.2.4.2.1 Collection

Towards the end of the first flowering season, in mid-February 2005, inflorescences from 10, seven and four control (unmanipulated) plants from the 450 m, 1070 m and 1300 m sites respectively, were counted and collected. Every bunch of inflorescences from each individual plant was placed in a clay pot, which had a 1 mm polyester mesh bag attached to it from the inside. Clay pots enabled the exchange of humidity between the contents of the pot and the surrounding soil to avoid desiccation of the insects and to provide protection from excess moisture. The mesh bags were stitched to a cone shape with two open ends. The narrower end was threaded through the hole at the bottom of the clay pot and knotted (Figure 4.2) to anchor the bag. Through the open top part, a small amount of potting mix was inserted and then the inflorescences. The potting mix was sterilized before being placed inside the pots in order to prevent the entry of insects from other sources. The potting mix was composed of sifted bark, peat and Vermiculite, in equal ratios. This combination enabled water absorbance (bark and peat), softness (peat) and air spaces (vermiculite). The potting mix was inserted to keep insects that leave their florets alive. Another knot was tied at the top part of the mesh bag to

prevent insects present in the florets from getting out of the bag. Each pot was labelled with the year, site and replicate. Pots were buried level with the soil surface so the inflorescence bag was level with or slightly above the soil surface. That way, all the insects were equally subjected to photoperiod, ambient air and humidity (Figure 4.3). At the 1070 m and 1300 m sites pots were buried no more than 1 meter away from their host plant while at the 450 m site pots were buried in a sheep-proof area, about 20 m away from the host plants. In that area small plastic square 'shade panels' were placed to the north side of the pots to protect them from overheating in direct sun.

Figure 4. 2 The nylon mesh bag and clay pot used for storing flowers and insect material in the field. The narrow end of the mesh bag was knotted outside the hole of the clay pot to anchor it**.**

Figure 4. 3 Small traps. Inflorescences stick out from the ground and are exposed to natural photoperiod, humidity and temperature

4.2.4.2.2 Emergence

Pots were left undisturbed in the field until mid October 2005. At that time, the top knots of the mesh bags were opened in each of the clay pots and the pots were covered with an emergence trap. Emergence traps were made from white plastic pots of the same diameter as the clay pots placed upside down on top of the clay pot and fastened in place with wire pegs that were stuck into the ground. Two plastic tubes were glued into holes in the side of each emergence trap (Figure 4.4a) each carrying a removable transparent vial for emerging insects to gather in. The transparent plastic 5 ml vials had open ends covered with 1 mm mesh to allow air to circulate through the vials and the emergence trap (Figure 4.4b and 4c, Figure 4.5). Vials were collected and capped and replaced with empty ones once a week from mid-October 2005 to early March 2006 in each of the three sites. Collected vials with insects inside were placed in the freezer overnight to kill the insects. The next day, insects were identified to species and counted, then preserved in labelled vials of 70% ethanol (Figure 4.6).

At the end of the season, when no more insects emerged from the pots, plastic pots were removed and knots were made in the top part of the mesh of each pot to allow insects to over-

winter another season.

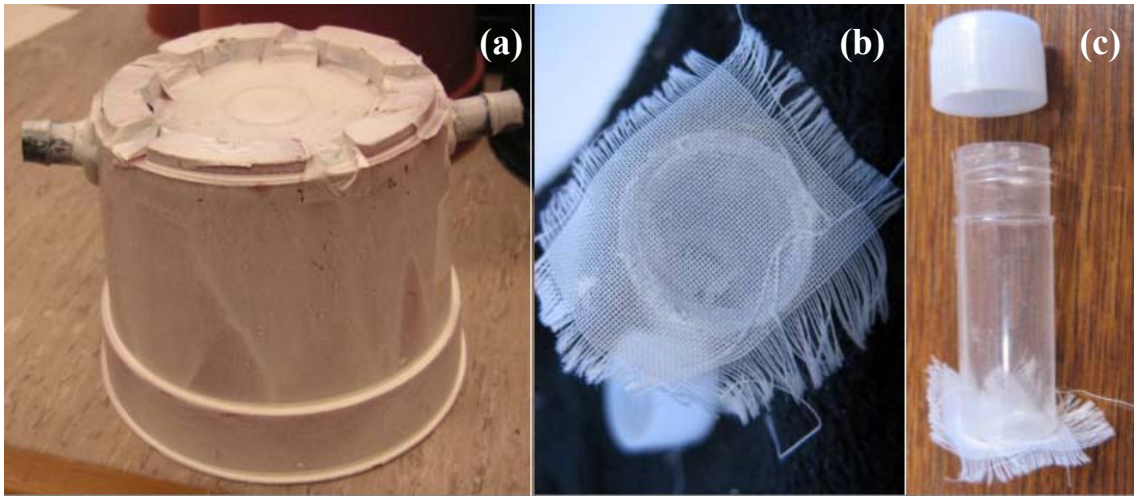

Figure 4. 4 (a) An emergence trap made from plastic pot; 5 ml vials for adult insect collection (b) lateral (c) ventral

Figure 4. 5 A pot at the 450 m site covered with an emergence trap and attached vials.

Figure 4. 6 Insects were preserved in vials of 70% ethanol labelled with the year of (larval) collection, the study site, the individual plant they emerged from and the date of (adult) insect collection.

4.2.5 *M. calamogonus* adult female dissections

Eggs of *M. calamogonus* have never been definitely identified in the wild or the lab, although White (1975) illustrated what he thought were such eggs, however, these turned out later to be eggs of *D. similis* (see discussion in McKone et al., 2001, pp. 95-96). Therefore in order to determine the egg size and shape, ten preserved female *M. calamogonus* collected in the large emergence traps on early November 2005 from 450 m site were checked for eggs inside their abdomen. Insects in ethanol turn slightly transparent and to identify whether females had eggs was easy without dissection. Ten females containing eggs were dissected under a light microscope, the length and the width of the largest eggs, which were close to the tip of ovipositor, were measured and all eggs seen were counted.

4.2.6 Data analysis

1. Predation levels of each seed predator species in each site were calculated as a proportion of all dissected florets containing each insect, plus the proportion of florets without the insect but containing species-characteristic signs of insect damage for the specific site.
2. The number of insects per plant for each of the three seed predators separately in the three years of emergence was calculated using the proportion of florets containing each insect (as in (1) above) multiplied by the mean number of florets per plant for 10 undisturbed *Chionochloa* plants at the three sites for each of the three seed predators separately according to the following equation:

$$N(\text{insect } i) = \frac{\text{inflorescence}}{\text{plant}} \times \frac{\text{spikelet}}{\text{inflorescence}} \times \frac{\text{floret}}{\text{spikelet}} \times P(\text{insect } i) \qquad (1)$$

   Where inflorescence/plant is the mean number of inflorescences per plant, spikelet/inflorescence is the mean number of spikelets per inflorescence, floret/spikelet is the mean number of florets per spikelet, and *P*(insect *i*) is the proportion of florets containing insect species *i*.
3. In order to test whether insect abundance differed in time (year) and space (site), I ran Mixed Effect Models for each insect separately, using R version 2.7.2 (R

Development Core Team, 2005). As fixed variables I used the interaction of 'year' and 'site' and as random variable I used 'site'. I utilized the binomial distribution with counting of damaged / undamaged florets per inflorescence as success / failure respectively. Finally, each model was back transformed using the logit function to get a mean abundance of insect per inflorescence and high and low Confidence Intervals.

4. In order to test for time dependence of emergence (phenology) of adult from the different species, repeated measures design was done for insect emergence from my large traps over time. The response variable used was calculated as the proportion of adult emergence (*P*) using the following equation:

$$Sin^{-1}\sqrt{P} \qquad (2)$$

Species were used as the subject and time (measured in Julian days) was used as within-subject, with trap number as Error.

4.3 Results

4.3.1 Flowering intensity

The southern hemisphere summer flowering season is from November to the following March. In the summer of the 2004/05 season, flowering was moderate with relatively few inflorescences (Table 4.1). Mean temperature of previous January-February at the 1070 m site was 11.3ºC, which is slightly colder than the mean average of January-February previous to this study, at 1996-2004 at this site (11.6ºC) (Kelly et al., 2008). In contrast, 2005/06 was a very high flowering year with many inflorescences at all three sites (see Table 4.1 for counts of inflorescences at the 450 m and 1070 m sites; personal observation for 1300 m site), flowering started relatively early, as the temperatures were comparatively high already in early summer (November 2005 had mean temperature of 8.4ºC at the 1070 m site, warmer than the average temperature for this month (7.6ºC from 1996-2004 at this site, (Kelly et al., 2008)). Mean temperatures of previous January-February were higher than usual and averaged 13.2ºC at the 1070 m site (Kelly et al., 2008). At the end of the flowering season, dry florets blow away from the tussocks with the wind and during this season florets left

tussocks relatively early, in late February. The following 2006/07 season was a very low flowering year (Table 4.1), and this was accompanied by relatively low temperatures in December (7.1ºC, mean temperature for the 1070 m site during 1996-2004 is 9.9ºC) and January (10.3ºC, mean temperature for the 1070 m site during 1996-2004 is 11.5ºC), although it was slightly warmer in February 2007 (11.3ºC, mean temperature for the 1070 m site during 1996-2007 is 11.7ºC) but still not as warm as usual (Kelly et al., 2008).

Table 4. 1 Mean number of inflorescences per tussock, number of tussock counted over three years in three altitudes. Inflorescences counted in mid-January – early February of each of three consecutive years.

Site	Year	Mean inflorescences per tussock	Number of tussocks counted
450 m	2004/05	100.06	65
	2005/06	106.37	88
	2006/07	12.41	90
1070 m	2004/05	6.90	60
	2005/06	59.40	85
	2006/07	1.45	90
1300 m	2004/05	2.82	39
	2005/06	18.54*	*
	2006/07	0.21	60

* not measured in that year, so estimated from mean increase over 2004/05 from 1070 m (8.61-fold, this table) and 1540 m *C macra* (4.54-fold, D. Kelly pers. comm.) giving an estimated 6.58-fold increase at 1300 m (see Methods).

4.3.2 Predation levels

The three seed predators were present at the three altitudes in different ratios (Table 4.2). In 2004/05 at the lower site, *M. calamogonus* was the dominant species, and it was responsible for about two thirds of all seed predation. At the 1070 m site, *D. similis* was responsible for about half of all seeds predated and at the 1300 m site, *E. chionochloae* preyed on four out of five seeds lost to predators. About one third of the total florets dissected during this season were preyed by one of the three seed predators (Table 4.2). The 2005/06 season was a high flowering year with the third highest flowering intensity during the last 21 years (Kelly et al., 2008) but total predation levels were not especially low (Table 4.2). *E. chionochloae* was responsible for more than half the seeds lost to predators at the 450 m, about two thirds at the 1070 m and four out of five seeds of the total number of seeds predated at the 1300 m site. *M. calamogonus* and *D. similis* were present in a smaller percentage of florets over the different elevations in 2005/06 in comparison to 2004/05, and mostly also lower than during 2006/07. The 2006/07 season was a low flowering year **and** had the highest predation levels of all three years at all three sites where out of all florets dissected from all sites, almost two thirds had insects in them or insect damage (Table 4.2). Against my predictions, at the 450 m

site *E. chionochloae* was responsible for predation of three out of five seeds lost to predators whereas *D. similis* was responsible at the 1070 m site for almost every seed predated and at the 1300 m site three out of five seeds predated. Overall, predation by the three insects differed across sites and years (see next section for statistics). *D. similis* peaked (in terms of percentage of florets damaged) in the year after a high flowering year and was most common at the upper two altitudes. *E. chionochloae* peaked in the high flowering year (2005/06) at the 1070 m site but was also very common at the other two altitudes in lower flowering years, for example, it was very abundant at the 1300 m site during the 2004/05 moderate flowering year and at the 450 m site during the 2006/07 low flowering year. *M. calamogonus* decreased with altitude and did not change as much as the other two insects from one year to another (see Figure 4.7 for mean insects per inflorescence).

Table 4. 2 Predation levels (percent of florets) by the three seed predators over three different years in three elevations at Mt. Hutt.

Site	Predator species	2004/05	2005/06	2006/07
450 m *(C. rubra)*	*M. calamogonus*	21.69	12.83	13.00
	D. similis	5.56	4.91	5.85
	E. chionochloae	6.62	20.08	27.43
	Total	**31.31**	**37.81**	**46.28**
1070 m *(C. pallens)*	*M. calamogonus*	5.11	4.41	3.25
	D. similis	14.2	5.50	84.70
	E. chionochloae	7.04	26.37	1.27
	Total	**26.35**	**36.28**	**89.22**
1300 m *(C. macra)*	*M. calamogonus*	2.16	0.14	1.90
	D. similis	6.04	5.38	29.98
	E. chionochloae	38.42	21.98	14.90
	Total	**46.62**	**27.5**	**46.78**
	Grand mean	**34.76**	**33.86**	**60.76**

4.3.2.1 Proportion of predated florets per inflorescence

The proportion of florets per inflorescence which were damaged by *M. calamogonus* was significantly greater at the lower site than at the two higher sites. Also the proportion of florets predated was significantly greater during 2004/05 flowering year in comparison to the following two summer seasons. There was also one significant interaction (the 2005/06 flowering year at the 1300 m site: Table 4.3, Figure 4.7).

There was no significant main effect of year for *D. similis*, but the proportion of florets per inflorescence which were damaged by *D. similis* was significantly greater at the 1070 m site than at the 450 m site. There were three significant interaction terms, two of which reflected

the very high proportion of florets attacked in the third season at the upper two sites (Table 4.3, Figure 4.7).

The proportion of florets per inflorescence which were damaged by *E. chionochloae* showed significant differences among years and among sites (peaking at 1300 m), plus three significant interaction effects (Table 4.3, Figure 4.7).

Table 4.3 Generalized linear mixed effect model and mean proportion of predated florets (± Confidence Intervals (CI) 95%) of predated florets, run on insect predation per inflorescence with 'year' and 'site' as predictors ('site' is a random effect), using a binomial error distribution and logit link function. Significant values are in bold.

Species		Mean	Low CI	High CI	Estimate	SE	z value	Pr(>|z|)
M. calamogonus	2005: 450 m	0.217	0.192	0.245	-1.284	0.079		
	2006	0.126	0.106	0.150	-0.649	0.127	-5.096	**<0.001**
	2007	0.130	0.108	0.156	-0.617	0.133	-4.624	**<0.001**
	1070 m	0.194	0.155	0.239	-1.426	0.137	-10.393	**<0.001**
	1300 m	0.074	0.045	0.120	-2.530	0.273	-9.274	**<0.001**
	2006 : 1070 m	0.571	0.465	0.671	0.286	0.217	1.316	0.188
	2007 : 1070 m	0.484	0.354	0.617	-0.064	0.274	-0.233	0.816
	2006 : 1300 m	0.111	0.016	0.491	-2.083	1.042	-1.999	**0.046**
	2007 :1300 m	0.620	0.416	0.789	0.490	0.423	1.159	0.246
D. similis	2005 : 450 m	0.056	0.043	0.072	-2.833	0.143		
	2006	0.048	0.036	0.064	-0.146	0.210	- 0.696	0.486
	2007	0.059	0.044	0.078	0.055	0.210	0.263	0.793
	1070 m	0.781	0.722	0.830	1.270	0.160	7.953	**<0.001**
	1300 m	0.522	0.418	0.625	0.089	0.214	0.418	0.676
	2006 : 1070 m	0.244	0.164	0.346	-1.133	0.254	-4.470	**<0.001**
	2007 : 1070 m	0.962	0.939	0.976	3.220	0.245	13.143	**<0.001**
	2006 : 1300 m	0.502	0.353	0.651	0.008	0.313	0.024	0.981
	2007 : 1300 m	0.863	0.785	0.916	1.841	0.279	6.609	**<0.001**
E. chionochloae	2005 : 450 m	0.066	0.052	0.084	-2.646	0.131		
	2006	0.198	0.173	0.225	1.246	0.156	8.009	**<0.001**
	2007	0.274	0.244	0.307	1.673	0.154	10.846	**<0.001**
	1070 m	0.570	0.490	0.646	0.280	0.163	1.719	0.086
	1300 m	0.898	0.867	0.922	2.174	0.153	14.226	**<0.001**
	2006 : 1070 m	0.523	0.428	0.616	0.092	0.194	0.476	0.634
	2007 : 1070 m	0.025	0.012	0.052	-3.657	0.382	-9.582	**<0.001**
	2006 : 1300 m	0.113	0.080	0.158	-2.061	0.197	-10.473	**<0.001**
	2007 : 1300 m	0.050	0.034	0.074	-2.944	0.209	-14.106	**<0.001**

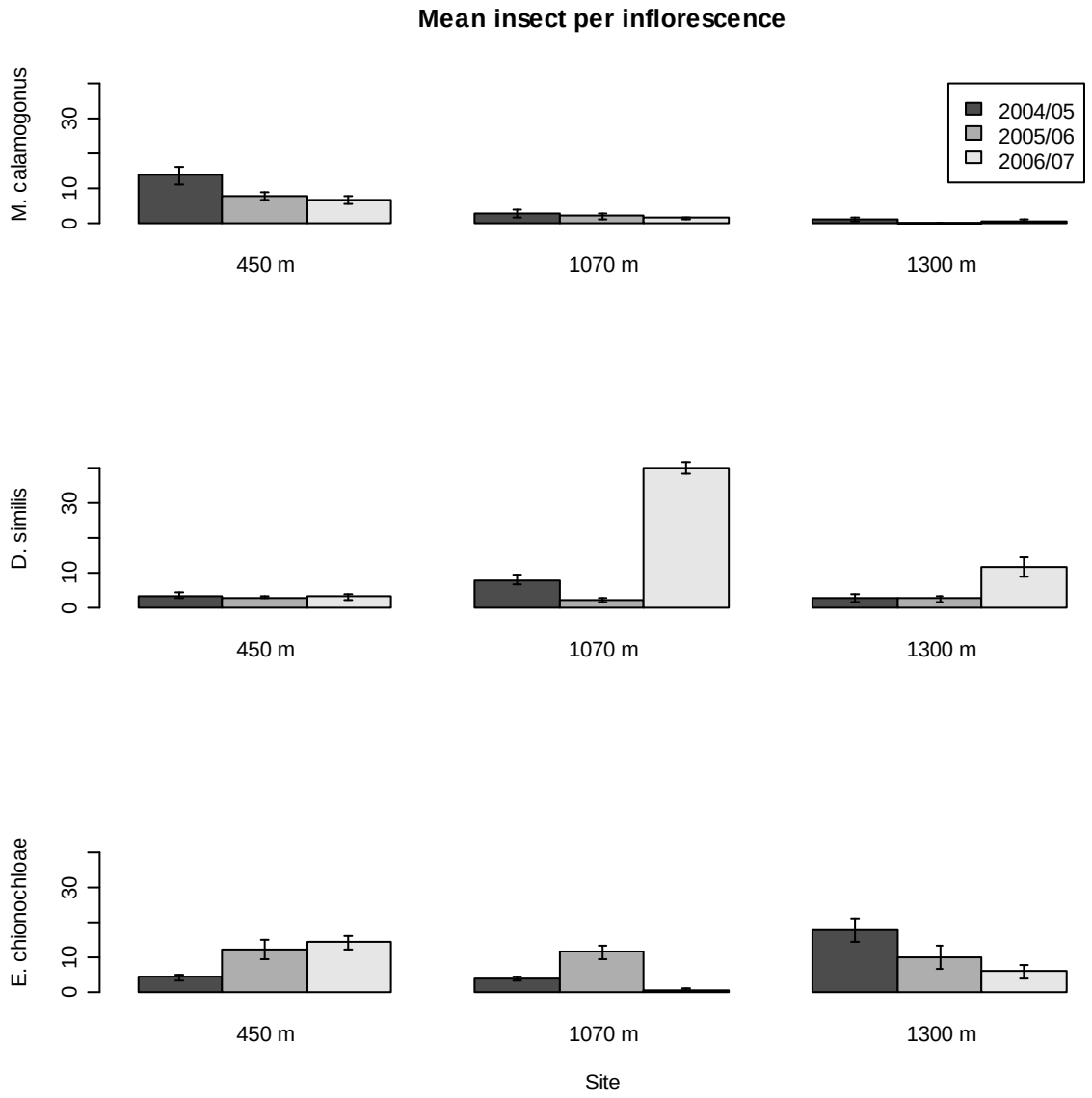

Figure 4. 7 Mean insect (± SE) per inflorescence over three year of study and three sites on Mt. Hutt.

4.3.2.2 Insects per plant

In the three years of emergence the number of insects per plant found at the three sites changed according to the number of florets available for the seed predators to consume (Table 4.4). For example, at the 1070 m site *D. similis* and *M. calamogonus* larvae per plant were respectively 35 and over 1900 times greater during the 2005/06 high flowering season than the 2006/07 low flowering season whereas *E. chionochloae* was over 4200 times greater during the 2005/06 high flowering season than the 2006/07 low flowering season (Table 4.4). The reduction of insect abundance per plant reduces with plant size (and elevation, as plants

are smaller in higher sites) for all species. Overall, *E. chionochloae* was 3 and 6 times more abundant than *D. similis* and *M. calamogonus* respectively at the 1070 m site. At the 1300 m site *E. chionochloae* was 4 and 55 times more abundant than *D. similis* and *M. calamogonus* respectively. There was no difference between *M. calamogonus* and *E. chionochloae* in abundance per plant at the 450 m site, but these two insects were about 3 times more abundant than *D. similis*.

Table 4.4 Estimated total number of larvae per plant in each year and mean number per plant over the three years of the three seed predators at Mt. Hutt over three elevations and three summer seasons

Site	Year	*M. calamogonus*	Mean (± SE)	*D. similis*	Mean (± SE)	*E. chionochloae*	Mean (± SE)
450 m	2005*	5755	3595±1618	1474	1143±482	1077.22	3062±2071
	2006*	4602		1761		7203.09	
	2007	429		193		906.03	
1070 m	2005	63	202±171	177	338±242	88.32	1206±162
	2006	543		815		3530.22	
	2007	0		23		0.82	
1300 m	2005*	7	3±2	19	38±28	118.78	167±112
	2006	3		93		380.51	
	2007	0		2		0.79	

* Number of Spikelets per Floret and Florets per Spikelet, which were used for the calculations in these tables (see section 4.2.6) were not measured in these years and therefore an average of previous and later years was used. These numbers change relatively little between years (Kelly et al., 1992).

4.3.3 Phenology of the seed predators

4.3.3.1 M. calamogonus

The number of adult *M. calamogonus* found in the large emergence traps during 2004/05 and 2005/06 summer seasons at each of the sites is shown in Figure 4.8. Other adults and larvae were also found in dissections during 2004/05 summer season, listed in Table 4.5 for each of the three sites. Larval phenology is shown in Figure 4.9 for 450 m and 1070 m sites.

Table 4. 5 Total number of insects found in dissections in the three sites during 2004/05 summer season at Mt. Hutt. There were no eggs of *M. calamogonus* or pupae of *E. chionochloae* found in these dissections.

Species	**Life Stage**	**450 m**	**1070 m**	**1300 m**
M. calamogonus	Larva	23	8	4
	Adult	68	26	3
D. similis	Egg	0	25	0
	Larva	2	139	3
	Pupa	6	170	0
E. chionochloae	Egg	7	192	500
	Larva	211	1408	3067

The first *M. calamogonus* adults caught at the beginning of the 2005/06 summer season are probably overwintering individuals from the previous flowering season. The first collection

from each of the traps contained many adults (mean of 75% of the total adults which were caught throughout the season), suggesting that they had started to be active before I placed these traps in early November. Very few individuals (<1%) were found in the large emergence traps between 22.11.05 and 26.01.06 (326-391 Julian days) at the 450 m site and then numbers caught increased again, which suggest that after this date most or all the adults are from the new generation (Figure 4.8). In addition, I found one adult in my small emergence traps at the 450 m site during the week of late November and early December 2006 (collecting emergence from flower material which had been sealed in a mesh bag since the previous autumn), which apparently confirms the ability of adults to survive over winter (the pupal stage is brief and occurs in autumn). There was a significant difference between the time of emergence of the two *M. calamogonus* generations (Table 4.6).

Table 4.6 Repeated Measures test for timing of adult emergence at the 450 m site during 2005/06 summer season. Species used in this model are the two different generations of *M. calamogonus* (coded as two different species for the analysis) and *E. chionochloae* (see Figure 4.8).

Error: Trap					
	df	SS	MS	F	P
Residuals	4	<0.001	<0.001		
Error: Within					
	df	SS	MS	F	P
Species	2	0.259	0.130	368.459	**<0.001**
Time	1	0.148	0.148	421.101	**<0.001**
Species x Time	2	0.059	0.030	84.117	**<0.001**
Residuals	260	0.091	0.000		

From the dissections of *M. calamogonus* females, I observed 245 small white rectangular eggs of which the largest 56 eggs were flat 0.4 × 0.6 mm on average. In the next collection which was in mid-November, females did not have any more eggs. That suggests that females laid their eggs around early November and the size of the eggs laid should be similar or slightly larger than the size I measured. Larvae were found mainly in the large emergence traps, whereas I found few more larvae from floret dissections (Table 4.5). The larvae are very mobile and probably many have left the florets after collection in the field and before dissections were done in the lab. No pupae were found in the florets or in the large traps at any of the sites.

4.3.3.2 D. similis

Adults which were found in the large emergence traps are presented in Figure 4.8 for both 2004/05 and 2005/06 flowering years at the 1070 m site. The first individuals of each summer season probably hatched during the previous summer and over-wintered as adults. Similar to *M. calamogonus*, current-season maturing larvae pupate and emerge rapidly in the autumn. At the 1070 m site, very few individuals of the total adults caught in the season (<1%) were found over 2004/05 between 21.01.05 and 02.03.05 (386-426 Julian days) and over 2005/06 between 05.01.06 and 19.01.06 (370-384 Julian days). This suggests that after these dates I was catching the new generation (Figure 4.8). As flowering started earlier during 2005/06 summer season and so did insect activity, there was a significant difference in the median emergence dates of first generation adults during 2004/05 (mean = 371.9 Julian days) versus 2005/06 (mean = 343.6, $t = 5.17$, d.f. = 16 emergence traps, $p < 0.001$).

Most adult and immature stages were found at the 1070 m site (Table 4.5). At the 1070 m site, unhatched eggs were not present in the florets later than mid-January 2005 but hatched eggs were found in the florets and glumes during the whole flowering season. Empty puparia, which were found in the florets until the last dissection batch, are brown and transparent and can be usually found in the florets at the end of the flowering season after pupae have emerged.

One adult *D. similis* was found in the small emergence traps during the 2006/07 summer season at 1070 m during mid-late January. This adult must have either emerged as an adult inside the bag the previous autumn and over-wintered in my traps, or spent the winter in diapause as a larva or pupa to emerge in the second summer. The former seems most likely since I have seen no evidence of any intact late-season pupae with diapausing larvae inside, whereas empty pupal cases are often seen (Table 4.5).

4.3.3.3 E. chionochloae

In order to test whether *E. chionochloae* adults found in the large emergence traps followed an altitudinal gradient I ran the same analysis of repeated measures describes in 4.2.7 (4) but I

used ‘site’ as a predictor rather than ’species’ (Table 4.7). Adults from the 1070 m site emerged significantly earlier than those from the 1300 m site (Table 4.7, Figure 4.8).

I found high numbers of *E. chionochloae* larvae in the dissected florets of all three years at all three sites until the last collection of florets around early March (Figure 4.9). This seems to be because the larvae remain in the florets and wait for the florets to fall off the plant in autumn. Therefore, the cumulative graphs show less clearly when larvae are present and feeding than for *D. similis* and *M. calamogonus* whose larvae leave the florets after feeding – any late season sample will always find *E. chionochloae* larvae, and if more late samples are taken, the cumulative graph will be skewed later in the season.

Table 4.7 Repeated Measures test for the timing of emergence of *E. chionochloae* adults emerging at the 1070 m site and at the 1300 m site during 2004/05 summer season. (See Figure 4.8).

Error: Trap					
	df	SS	MS	F	P
Site	1	0.350	0.350	1.623	0.227
Time	1	0.370	0.370	1.718	0.215
Residuals	12	2.587	0.216		
Error: Within					
	df	SS	MS	F	P
Species	1	3.277	3.277	43.350	**<0.001**
Time	1	148.780	148.780	1967.877	**<0.001**
Species x Time	1	0.858	0.858	11.351	**<0.001**
Residuals	429	32.434	0.076		

4.3.3.4 M. calamogonus *vs.* E. chionochloae *450 m site*

Adults of *E. chionochloae* at the 450 m site emerged significantly later in the 2005/06 season than the first *M. calamogonus* generation (Table 4.6). Unfortunately, I could not compare mean emergence of *M. calamogonus* to either *D. similis* or *E. chionochloae* at the 1070 m site as the number of adults caught was too small to analyse.

4.3.3.5 D. similis *vs.* E. chionochloae *1070 m site*

Adults of *D. similis* first generation were present at the 1070 m site significantly earlier than adults of *E. chionochloae* in both the 2004/05 (Table 4.8, Figure 4.8) and the 2005/06 (Table 4.9, Figure 4.8) summer seasons.

Table 4.8 Repeated Measures for adult emerging at the 1070 m site during 2004/05 summer season. Species used in this model are *E. chionochloae* and the first generations of *D. similis* (see Figure 4.8).

Error: Trap					
	df	SS	MS	F	P
Residuals	14	32.447	2.318		
Error: Within					
	df	SS	MS	F	P
Species	1	1.887	1.887	11.806	**<0.001**
Time	1	142.328	142.328	890.362	**<0.001**
Species x Time	1	5.270	5.270	32.967	**<0.001**
Residuals	582	93.035	0.160		

Table 4.9 Repeated Measures for adult emerging at the 1070 m site during 2005/06 summer season. Species used in this model are *E. chionochloae* and the first generation of *D.* (see Figure 4.8).

Error: Trap					
	df	SS	MS	F	P
Residuals	4	<0.001	<0.001		
Error: Within					
	df	SS	MS	F	P
Species	1	0.025	0.025	72.128	**<0.001**
Time	1	0.143	0.143	407.073	**<0.001**
Species x Time	1	0.018	0.018	51.523	**<0.001**
Residuals	152	0.053	0.000		

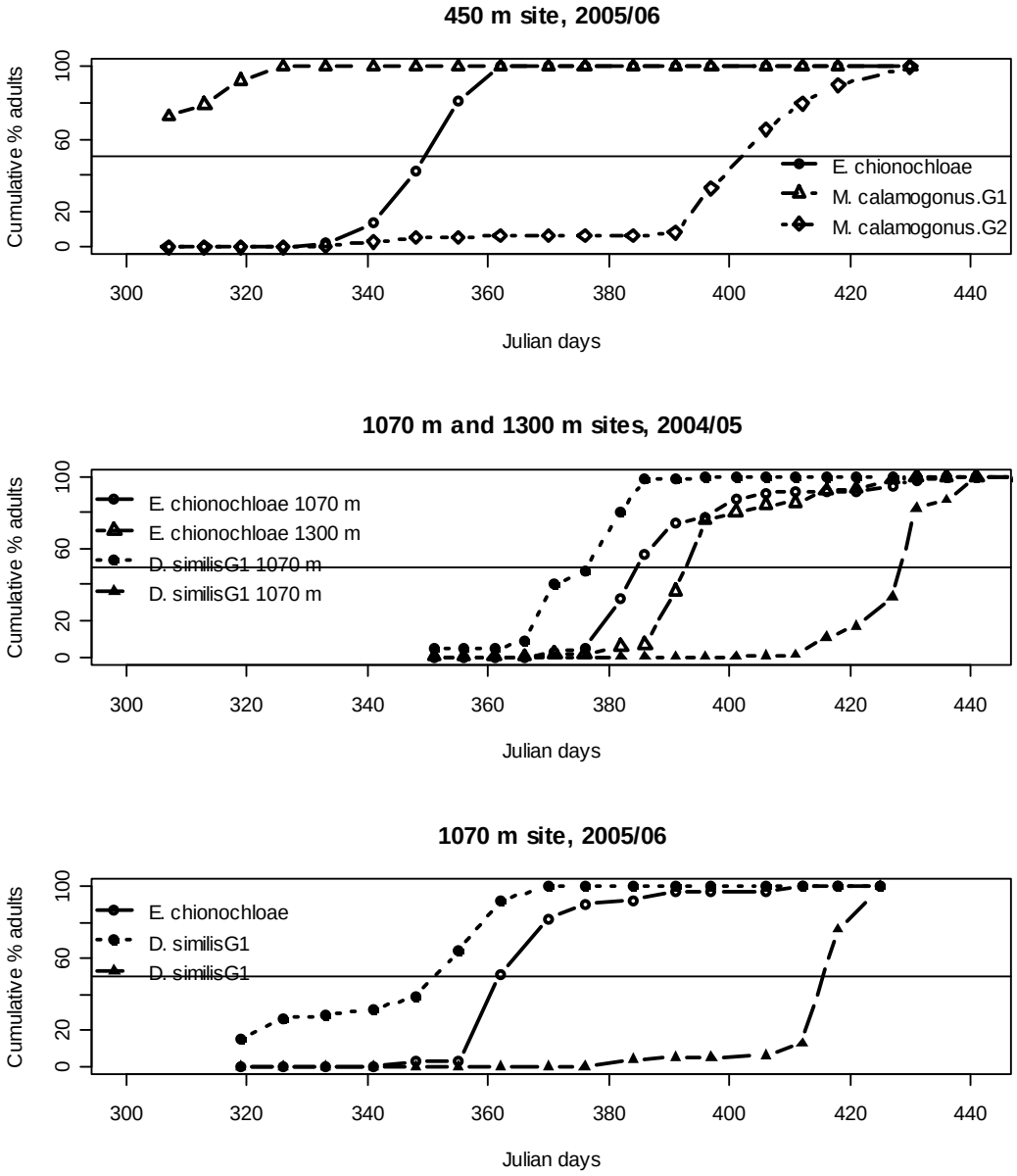

Figure 4. 8 Cumulative percentage emergence of *M. calamogonus*, *E. chionochloae* and *D. similis* adults at the different sites and years collected from large traps. Dates are in Julian days where 300 = 27 October and 440 = 15 March 2005. G1 and G2 represent Generation 1 and Generation 2 respectively. Site/species combinations which are not presented had too few data.

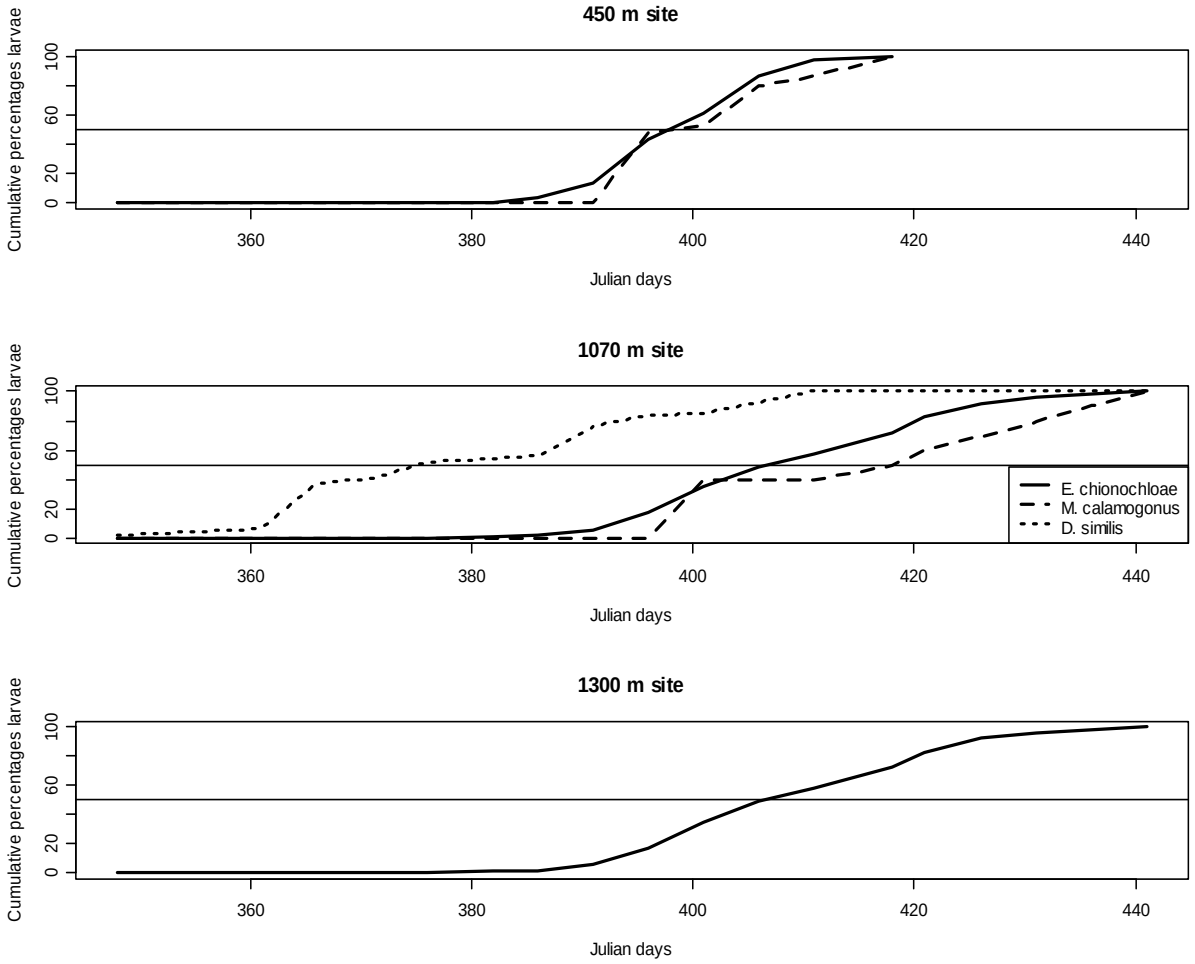

Figure 4. 9 Cumulative percentages of larvae found in floret dissections during 2004/05 summer season. Dates are in Julian days where 360 = 25 December 2004 and 440 = 15 March 2005. Site/species combinations not presented had too few data (see Table 4.5).

4.4 Discussion

4.4.1 Mast seeding and percentage predation

There is clear evidence that masting in *Chionochloa* results in strong predator satiation (Kelly & Sullivan, 1997; Kelly et al., 2008). Total predation by all three herbivores reduced from more than 70% in low flowering years to less than 10% in some high flowering years in a 20 year dataset (Kelly et al., 2008). The 2004/05 season was a moderate flowering season but certainly not one of the lowest seen (Kelly et al., 2008). Predation levels by all three insects combined averaged 35% across all sites, which is not particularly high relatively to some other moderate flowering years (see Figure 1b in Kelly et al., (2008)). On the other hand, 2005/06 was one of the highest flowering seasons in the last 22 years, but predation levels were not especially low (overall mean 34%) largely because of the increased incidence of *E. chionochloae*. The 2006/07 summer season, which was a very low flowering year suffered

heavy predation levels by the three species (60% mean across all sites), especially at the 1070 m site with 89% predation, most of which are attributed to *D. similis*. Looking at the data on a per site basis, only the 1300 m site behaved as predicted in the three years of study. That is, higher predation levels in the two low flowering years and lower predation levels in the high flowering year. Predation levels by *E. chionochloae*, which can enter prolonged diapause if flowering intensity is not especially high, were not only synchronized with the 2005/06 high flowering year and high predation levels were seen in some low flowering years by this insect. These data firstly suggest that a longer term dataset is necessary in order to follow trends of predator satiation in this system. Secondly, there may be other factors affecting insect emergence and predation levels other than flowering intensity. Such factors might be related to climate (i.e., cold winters may have killed more overwintering adults (*D. similis* and *M. calamogonus*) or diapausing larvae (*E. chionochloae*); low mean temperatures in October and high mean temperatures in November of the same season may change flowering intensity (Kelly et al., 2008) but this temperature cue might not have been detected by the insects; plant or insect physiology may vary (e.g., insufficient feeding by *E. chionochloae* might result in shorter length of diapause); predation by natural and introduced enemies and other biotic and abiotic factors.

4.4.2 Interspecific competition and resource partitioning

Interspecific competition between phytophagous insects was not always considered important in shaping community structures, abundance or distribution (Hairston et al., 1960; Lawton & Strong, 1981; Schoener, 1982). However, recently more papers show that interspecific competition is important for insects, reviewed in both Denno, McClure and Ott (1995) and Kaplan and Denno (2007). Competition theory suggests that the interactions between two organisms, which struggle for resources, would be more extreme if their abundance is high or if they share spatiotemporal co-occurrence and ecological similarities, such as feeding guild or phylogenetic relatedness (Kaplan & Denno, 2007). Herbivores that feed at different times of the flowering season and/or use different parts of the plants for food are considered to exploit different niches and therefore experience reduced competition (Schoener, 1974; Connell, 1980). Although the three seed and flower predators of *Chionochloa* do not share phylogenetic relatedness, they do share a similar feeding guild; however each is feeding on slightly different part of the florets and therefore partitions the food resources. According to McKone et al., (2001), *M. calamogonus* feed on the anthers and palea and other floral parts and do not need to wait for the ovary to develop into seed. *D. similis* seem to feed mainly on the undeveloped anthers at the beginning of the flowering season, while *E. chionochloae* feed

on the developing seed and therefore must feed later in the season. The importance of niche differentiation is recognized as vital in maintaining biodiversity (Chesson, 2000), but a variety of indirect interactions can result if consumer species share their resources (Levine, 1976; Holt, 1977; Vandermeer, 1980; Abrams, 1986). On the other hand, species from different phylogenetic relatedness which are occurring in the same environments will be forced by the same selective pressures and therefore may develop similar characteristics to cope with that environment and hence be more similar to each other than other species in different environments (Leibold & McPeek, 2006).

The difference in insect abundance can be accounted for by other reasons over the three sites: Hay et al., (2008) suggested that *D. similis* preferred to feed on *Chionochloa pallens* whereas *E. chionochloae* was more abundant on *C. macra*. Although I could not test for insect food preference in this study (because I only had one site per plant species), *D. similis* was significantly more abundant at the 1070 m site than any other site, where *C. pallens* is dominant (Table 4.3). It is not possible to conclude from my data whether *E. chionochloae* have any preference for specific plant species as it did not have a significant difference in its abundance between sites (plant species). *M. calamogonus* clearly was more common at lower elevations – where *Chionochloa rubra* (its most common host) grows and mast seeding is less extreme (Sullivan & Kelly 2000). Moreover some food is usually available even in low flowering years in this site (Table 4.1), which may have to do with enriched soil from sheep droppings and therefore availability of resources to the plants.

4.4.3 Insect abundance among sites and years

The percentage of florets attacked by *M. calamogonus* significantly differed between sites (plant species) and decreased with increasing elevation (Tables 4.2 and 4.4). *M. calamogonus* was the earliest seed predator in the lower site, where flowering starts earlier than in the other higher cooler sites (Figure 4.8 and 4.9). Therefore, it may have an advantage of feeding readily even when at high predation levels in low flowering years (Table 4.2). Because this insect species has the advantage of feeding first at lower elevations (Figure 4.9) and because of its destructive feeding behaviour, in agreement with Cone (1995), I suggest that it may exert selective pressure on *D. similis* and *E. chionochloae* to shift to higher elevations where competition with *M. calamogonus* is less intense. At the 450 m site, *D. similis* was found in low percentages (Table 4.2) whereas some years had very high percentages of *E. chionochloae* abundance. It is therefore not clear what the interactions between *E.*

chionochloae and *M. calamogonus* are like. *D. similis* might have an advantage over *E. chionochloae* at higher elevations as the larvae are feeding earlier (Figure 4.9) and feed on flowers rather than developing seeds (McKone et al., 2001). In low-flowering years, such as 2006/07, both *M. calamogonus* and *D. similis* might put selective pressure on *E. chionochloae* as the earlier two insects feed extensively on the florets and probably decrease the availability of food for *E. chionochloae*. However in some low flowering years the low percentages of predation by *E. chionochloae* is probably a result of many insects staying in diapause, although some low flowering years suffered high percentages of predation by this insect (e.g., 2006/07 at the 450 m site, 2004/05 at the 1300 m site) so the trends are probably caused by a combination of factors affecting emergence from diapause (see Chapter 6). In high flowering years, the populations of *D. similis* and *M. calamogonus* were usually rather small relative to the amount of food available (Table 4.4). That may have to do with their adult population, which is limited by the numbers that emerged the previous flowering season (and survived subsequent winter mortality). Therefore, in a high flowering year, they are limited in their numerical increase by the maximum number of eggs each female is capable of producing. In addition, *D. similis* has a very large egg relative to the size of the female (McKone et al., 2001) and each female can probably only lay a few eggs at a time. Therefore *D. similis*'s fecundity is thought to be rather low in comparison to *M. calamogonus* and *E. chionochloae*. The high fecundity of *E. chionochloae* and its emergence from prolonged diapause suggest that they may take advantage of the small numbers of the other competitors in high-flowering seasons to prey upon large number of florets and maximize their fitness. McKone et al., (2001) reported a smaller density of *E. chionochloae* when a larger density of *D. similis* occurs. In the present study this trend can be seen within sites at the 1070 and 1300 m sites during the lowest flowering year 2006/07 and at the lower site during 2005/06 and 2006/07 flowering years (Table 4.2). Nevertheless, in a high flowering year (2005/06), higher density of *E. chionochloae* occurs with smaller density of *D. similis*.

Stable coexisting species ('densities of the species in the system do not show long term trends… [but if] densities do get low, they tend to recover' (Chesson, 2000)) require that each of the coexisting species will respond to ecological heterogeneity in different ways. That difference in response would usually result from trade-offs of the species abilities to interact with a variety of environmental characteristics (Chesson, 2000). It is possible to refer to the *Chionochloa* system and its herbivorous as a stable one. Even though the densities of *E. chionochloae* seem less stable than those of *M. calamogonus* and *D. similis* (Table 4.4) and, although there are fewer *E. chionochloae* adults seen (and hence lower recruitment of larvae)

in some low flowering years, the population can still be considered as a stable one, where most larvae would probably be still alive and in diapause.

It is established that mast seeding places a selective pressure on the seed predators to cope with the lack of resources during low flowering years. *D. similis* and *M. calamogonus* may feed on other species of grasses from other families (Hudson, 1928; Cone, 1995) in low flowering years (although these other grasses are rare in the Mt. Hutt area). However, *E. chionochloae* has the ability to enter prolonged diapause (Chapter 2), which might be influenced not only by the actual lack of food but also by other factors such as inter-specific competition. Hence the response of the different species to the same environmental characteristic (variable food supply) is different and in spite of potential interspecific competition, these three herbivores coexist at many sites (White, 1975; McKone et al., 2001).

4.4.4 Phenology and altitude gradient

Phytophagous insects, which are usually sensitive to changes in temperature, may alter their phenology according to the conditions they face (Battisti et al., 2005; Speight et al., 2008). It was shown that the phenology of *E. chionochloae* is following an altitudinal (temperature) gradient where insects emerge earlier in the season at lower (warmer) elevations and later at higher (cooler) elevations. These findings support other studies overseas (e.g., the pine processionary moth (Battisti et al., 2005)). At the 450 m site it was shown that *M. calamogonus* was the first insect to be active, whereas *D. similis* was earlier at the 1070 m site.

There was a disagreement between Cone (1995) and Sullivan (1993) regarding which insect species emerges first, *M. calamogonus* or *D. similis*. Cone sampled in relatively lower elevations (ranging from 470 m to 800 m elevation at Mt. Hutt) and concluded in agreement with White (1975) that *D. similis* is present earlier than *M. calamogonus*. However, Sullivan sampled in various higher places including at 1060 m Mt. Hutt and concluded *M. calamogonus* was the first insect present (Sullivan, 1993; Cone, 1995). From my data, I cannot determine which of these claims is correct as I found *M. calamogonus* first at lower elevations and *D. similis* first in higher ones. Plant species may be a factor affecting the timing of emergence of the insects. For example, *Chionochloa rubra*, which was found to have particularly high levels of *M. calamogonus* predation (Sullivan & Kelly, 2000) is usually found in lower altitudes than *C. pallens* (*D. similis* prefered plant species, (Hay et al., 2008)) or *C. macra* (Table 1.1). The relatively high 1060 m site Sullivan (1993) sampled was dominated by *C. rubra*. I suggest that the alteration in timing of emergence between years of

the two herbivorous may be affected by inter-annual variations of biotic and abiotic conditions (e.g., plant species, plant resources, density of plants, and temperatures).

Although it was not possible to determine which of the insects, *M. calamogonus* or *D. similis* is active earlier, it is clear that adults of these two species are active earlier than *E. chionochloae* adults. This is influenced, at least in part, by the fact that *E. chionochloae* need to oviposit their eggs in florets with mature seeds, whereas *D. similis* and *M. calamogonus* largly eat anthers and floral parts. Florets damaged by *D. similis* and *M. calamogonus* might not develop into seeds, and therefore *E. chionochloae* will have fewer florets to consume during low flowering years when resources are limited. Consequently, it may be that *D. similis* and *M. calamogonus*, together with the unpredictable food supply of the plant, have a synergetic effect, which placed a selective pressure on *E. chionochloae* to enter extended diapause during low flowering years and emerge in high flowering years. The insects that emerged from diapause during low flowering years will be selected to forage for food in places where the other two insects are less abundant (e.g. at higher altitude). Few studies dealt with diapause or dormancy which is driven from competition. For example, Ellner (1987) presented a theoretical model of seed germination and concluded that competition may favor dormancy. However, he studied populations of siblings, which are seeds from the same mother plant that should germinate in the same year and therefore his model deals with intra-specific competition and not inter-specific competition. Annila (1981) studied different seed and cone insect species in Norway spruce *Picea abies* which compete for resources and also enter prolonged diapause, however each species has some differences in their diapause (Hanski, 1988) and it was not mentioned whether inter-specific competition was driving insects to prolong their diapause or whether other factors caused diapause to be selected. I failed to find other studies in the literature dealing with diapause that is driven from inter-specific competition.

4.4.5 Life strategies of each predator

McKone et al., (2001) suggested that *D. similis* is satiated by mast seeding more easily than *M. calamogonus* and *E. chionochloae*. In Table 4.4, I averaged the number of insects per tussock over three years for each of the elevations. This absolute abundance of each insect per inflorescence is influenced by flowering intensity and can be a relatively easy way of measuring insect abundance. At the 450 m site, on average *M. calamogonus* was more abundant than *E. chionochloae* and *D. similis*, whereas at the 1070 m and 1300 m sites on

average *E. chionochloae* was far more abundant than the other two insects. *E. chionochloae* had the highest mean in the two higher sites because it was usually (but not always) more abundant (as a percentage of florets) in the high flowering years and hence could attack a large number of predated florets, whereas *D. similis* was usually more abundant (percentage-wise) in the low years when there were fewer florets available to attack. White (1975) and McKone et al., (2001) suggested that *M. calamogonus* is more successful (better competitor) than *D. similis* in terms of increase in numbers in high flowering years. However, calculating abundance of damaged florets but not of total insects may skew the results because of the differences in the feeding behavior of the three insects. According to Cone (1995, p. 213), each *M. calamogonus* larva consumes about 17 florets, and *D. similis* about 3.2 florets per larva, while *E. chionochloae* larvae consume only 1.66 each.

D. similis however is using a different strategy to *M. calamogonus*. This is mainly because of the probable low fecundity of *D. similis* which has a relatively large egg compared to the female (McKone et al., 2001). *D. similis* maintains a steady population even in the low years although they have low population growth rates and as a consequence they are relatively small in numbers. The larvae are relatively large and mobile and feed before *E. chionochloae* does; therefore there are always some florets to consume which probably increase their survival rates. The steady population size of *D. similis* may have increased the risk of this species being attacked by host-specific parasitoids (see Chapter 5).

In contrast to the large eggs and low fecundity of *D. similis*, *M. calamogonus* has large numbers of smaller eggs per female. McKone et al., (2001) described three 1.53 × 0.28 mm green eggs found in late December within the florets of *Chionochloa rigida* from Central Otago which they suspected belong to *M. calamogonus*. These eggs are a different size and colour to the ones I dissected out from female's abdomens (which were white, 0.6 × 0.4 mm), which raises doubts about whether the Central Otago eggs were those of *M. calamogonus*, however without seeing laid *M. calamagonus* eggs there is no way to be sure of their final size or colour. The relatively large number of eggs each *M. calamogonus* female can oviposit may give it a competitive advantage over *D. similis*, especially as they both feed at a similar time, at the beginning of the season. The larvae of *M. calamogonus* are much larger and more mobile than those of *D. similis* and their destructive feeding behavior prevents *D. similis* feeding on the same florets afterwards. It appears that in low elevations, *M. calamogonus* is more successful than *E. chionochloae* (Table 4.4). McKone et al., (2001) re-examined White's (1975) data and concluded that *M. calamogonus* was more abundant relative to *D.*

similis in high flowering years than it was in lower ones. The average number per plant in my study across three years at the 450 m site supports this conclusion as well as the number of insects per plant for each year, which is larger in high flowering years than in lower ones (Table 4.4). I conclude that *M. calamogonus* is more abundant than *D. similis* and *E. chionochloae* at the low elevations and that *E. chionochloae* is more abundant than *D. similis* and *M. calamogonus* at the higher elevations mostly in high flowering years. However, *D. similis* has a different strategy of survival and a more stable population size.

To conclude, timing of emergence of the three seed predators differs with plant development and across elevations. *E. chionochloae* enters prolonged diapause which sometimes enables it to emerge in relatively low numbers in low flowering years but in high numbers in high flowering years. *D. similis* predation levels (as a percentage of florets) apparently decrease with increasing flowering intensity (i.e. it shows predator satiation). *M. calamogonus* has a steady population size at lower sites but low predation levels at higher sites no matter whether these had a high or a low flowering intensity. One adult *D. similis* and one adult *M. calamogonus* overwintered in my small emergence traps which confirmed that they have simple dormancy probably as adults. I suggest that *D. similis* and *M. calamogonus* together with the unpredictable food supply of *Chionochloa* play an important role in the selection for diapause of *E. chionochloae*. I suggest that by having diapause, *E. chionochloae* is a better competitor than *D. similis* and *M. calamogonus* at the higher elevations, whereas *M. calamogonus* is a better competitor at the lower elevations, although all species co-exist in the system at a wide range of South Island sites (McKone et al., 2001).

Introduction to Chapter 5

Several species of parasitoids from *Megacraspedus calamogonus*, *Diplotoxa similis* and *Eucalyptodiplosis chionochloae* were discovered during this study. This chapter describes the different species of parasitoid for each of the seed / flower predators and gives some information on their parasitism rate. *Megacraspedus calamogonus* endures a heavy load of parasitoids of which three are hymenopteran and one is dipteran: 1. *Zealachertus tortriciphaga* Berry (Hymenoptera: Chalcidoidea: Eulophidae); 2. *Diadegma* sp. (Hymenoptera: Ichneumonidae: Campopleginae); 3. *Dolichogenidea* sp. (Hymenoptera: Braconidae: Microgastrinae); 4. *Uclesiella* sp., (Diptera: Tachininae: Voriinae). *Diplotoxa similis* is probably parasitized by one hymenopteran species, from the genus *Callitula* (Pteromalidae: Pteromalinae) which parasitized about 1% of the larvae. *Eucalyptodiplosis chionochloae* is parasitized by two hymeopteran parasitoids: *Gastrncistrus* sp. (Pteromalidae: Pireninae) and *Zelostemma chionochloae* (Platydastridae: Platygastrinae) and suffered a relatively high parasitism rate of 41%.

All these species of parasitoids were either not known before this study (*Zelostemma chionochloae*, *Dolicogenidea* sp. *Uclesiella* sp., *Calitulla* sp.), known only to family (*Zealachertus tortriciphaga*) or genus (*Gastrancistrus* sp., *Diadegma* sp.). This is the first study which attempts to classify the species which are present in the *Chionochloa* system.

My contribution to this paper involved: (i) running all the technical work and insect rearing, (ii) writing the manuscript, and (iii) running data analysis, calculating the results and constructing the graphs. Eckehard Brockerhoff advised me regarding the host's rearing which led to the collection of adults of these parasitoid species. Both Dave Kelly and Eckehard Brockerhoff were involved in writing the manuscript and gave valuable comments to improve it.

5. The parasitoids of *Chionochloa* (snow tussock) seed predators

5.1 Introduction

Chionochloa is a genus of perennial long-lived tussock grasses which show pronounced mast seeding (i.e., synchronous highly variable seed production among years by a population of plants (Kelly, 1994)) (Kelly et al., 1992; Kelly et al., 2000; Schauber et al., 2002). *Chionochloa* species suffer high levels of pre-dispersal seed and flower predation (hereafter "seed predation") by three herbivorous insects (White, 1975; Kelly & Sullivan, 1997; Sullivan & Kelly, 2000; McKone et al., 2001; Kolesik et al., 2007). These are a chloropid fly (*Diplotoxa similis* Spencer 1977), a gelechiid moth (*Megacraspedus calamogonus* Meyrick 1885) and the recently described cecidomyiid fly *Eucalyptodiplosis chionochloae* (Kolesik et al., 2007). The extreme mast seeding of *Chionochloa* is thought to be an adaptive response to the heavy seed predation by seed predators (Kelly & Sullivan, 1997; Rees et al., 2002), which cause great reductions in seed output. In particular, *Eucalyptodiplosis chionochloae* was thought to use sophisticated life history strategies (i.e., prolonged diapause) to reduce emergence in low flowering years, increasing pressure on the plants to have a more variable flowering strategy (Kelly et al., 2000; McKone et al., 2001; Rees et al., 2002). It is now confirmed that *Eucalyptodiplosis chionochloae* enters prolonged diapause for at least two years (Kolesik et al., 2007). The diapause by *E. chionochloae* may serve as a 'refuge' during low *Chionochloa* flowering years, which may maximize long-term levels of predation by *E. chionochloae* on *Chionochloa* seeds.

However, parasitoids may play an important role in controlling the densities of their hosts, as shown, for example, by successful biological control projects (e.g., Matsumoto et al., 2003 and references therein). If seed predators like *E. chionochloae* are kept rare by the actions of an effective predator or parasitoid, this would reduce the selective pressure on the host plants to maintain extreme mast seeding. Therefore studying the interactions between *Chionochloa* seed/flower predators and their parasitoids is important for understanding the system as a whole. Endoparasitoids are expected to synchronize their own life cycles better with those of their host than ectoparasitoids, particularly during the diapause stage (Tauber et al., 1986). If such synchrony of diapause does exist, predation of seeds by *Chionochloa* seed predators is expected

to be lower overall because the parasitoids are likely to kill a larger percentage of the seed predators than without such adaptation. However seed predators may escape parasitoids by shifting their phenology or by having a superior reproductive rate.

Previous studies of the *Chionochloa* system suggested there were up to six different species of parasitoids which attack the three seed predators (White, 1975; Sullivan, 1993; Cone, 1995; McKone et al., 2001; Kolesik et al., 2007). However, information on many of these is fragmentary or contradictory (described in detail in the methods).

Because the information in the literature regarding the parasitoid species of *Chionochloa* seed predators is incomplete and sometimes contradictory, we aimed to expand knowledge of the insects in this study system, identify the species where possible, and record the phenology and ecology of the different parasitoids in relation to their hosts. Understanding the biology and ecology of the natural enemies of *Chionochloa* seed predators is important both for cataloging the endemic biodiversity of New Zealand, and also for understanding the evolution of mast seeding in *Chionochloa*.

5.2 Methods

5.2.1 The host plant

Chionochloa is a genus of 25 species, of which 23 are endemic to New Zealand and the other two (*C. frigida* and *C. howensis*) are endemic to Australia and Lord Howe Island, respectively (Connor, 1967; Connor & Lloyd, 2004). The genus is especially common in the central South Island, where our study took place.

5.2.2 Parasitoids previously mentioned in the literature

1. White (1975) associated Eulophid wasps reared in the lab with *D. similis* as they emerged from *Chionochloa* flower heads at the same time. However, Cone (1995) and Sullivan & Kelly (2000) thought that these eulophid wasps reported by White (1975) were associated

with *M. calamogonus* rather than with *D. similis*. These wasps showed a wide range of pupal and adult sizes, but even the larger specimens were relatively small compared to the size of adult *M. calamogonus*, which may have contributed to the confusion about the identity of the host.

2. White (1975) reported larvae of a hymenopterous parasite (Ichneumonidae: Campopleginae, previously Ichneumonidae: Porizontinae) associated with *M. calamogonus* which he tentatively ascribed to the genus *Diadegma*. This wasp was not found by Cone (1995) despite her relatively intensive sampling programme.
3. Cone (1995) found cocoons of what was thought to be a braconid wasp from *M. calamogonus*.
4. Cone (1995) found pupae apparently associated with another eulophid wasp inside cocoons of *D. similis*, as well as four adult eulophid specimens, one of which was next to an empty *D. similis* cocoon. She suggested that these eulophid wasps emerge later in the season when no more *D. similis* emerge as these wasp specimens were found after the emergence of adult *D. similis*.
5. Both Sullivan (1993) and Cone (1995) found adults of a Pteromalid parasitoid from the genus *Gastrancistrus* outside *Chionochloa* inflorescences. Cone (1995) found *E. chionochloae* larvae in those inflorescences using dissections and suggested that *Gastrancistrus* females oviposited inside these *E. chionochloae* larvae. Kolesik et al., (2007) found these *Gastrancistrus* wasps to be apparently specific to *E. chionochloae*.
6. Kolesik et al., (2007) also reported an undescribed species of Platygastid wasp from *E. chionochloae*. Subsequently Buhl et al., (2008) formally described the Platygastrid wasp as *Zelostemma chionochloae*.

5.2.3 Study site

The two study sites are the 450 m site and 1070 m site from Mt Hutt descried in Chapters 2-4.

5.2.4 Bulk collections

To collect a large number of larvae for subsequent investigations of the emergence of adults, a bulk collection of 247 inflorescences of *C. rubra* was made in mid-November 2004 from the 450 m site. Another two bulk collections of inflorescences of *C. pallens* were made in mid-February

2005 (over 450 inflorescences) and late January 2008 (over 100 inflorescences) from the 1070 m site. A total of 314 larvae of *M. calamogonus* were collected from the inflorescences from the 450 m site in 2004 and placed into a container. These larvae were kept moist using wet paper towels until they made cocoons. However, some larvae dried out (despite our efforts to keep them moist) or were too small to pupate and only 73 of them made cocoons. All larvae collected were *M. calamogonus*, but the cocoons included different parasitoid cocoons, so each group of cocoons were separated according to their appearance to a different container. All cocoons were reared to adults and then sent for identification. Three puparia of *Uclesiella* sp., (Diptera: Tachininae: Voriinae) did not emerge but could still be identified and these were counted with the emerging adults.

Detailed methods for the bulk collections made at the 1070 m site in mid-February 2005 are given in Kolesik et al., (2007). In short, inflorescences collected were brought to the University of Canterbury (20 m a.s.l) and placed in 1-mm mesh white polyester bags that were then placed inside 5 mm wire mesh to prevent predation by mammals while allowing exposure to the natural photoperiod and humidity. This bulk collection was placed outside on campus under shrubs from mid-February 2005 to early October 2005. Inflorescences were then brought back to the lab and placed in emergence chambers and all insects which emerged were collected, counted and identified to species. When emergence stopped in late November 2005, the inflorescences were placed back in the bags outside for a second winter season. The same procedure of collecting emerging adult insects was used again from mid-October to mid-November 2006 and (after a third winter outside) from mid-October to mid-November 2007, and insects emerging from prolonged diapause during these years were collected.

The 2008 bulk collection from the 1070 m site was brought to the lab and placed in an emergence chamber made of pyramid-shape which consisted of a wooden frame from 2.5 cm x 2.5 cm timber stakes covered with white 1 mm polyester gauze from bottom to the peak of the pyramid (about 50 cm high). One of the bottom sides of the pyramid had a pocket which can be sealed, through which the inflorescences were inserted to the pyramid space. Emerging insects were funneled into a jar with 70% ethanol which was replaced once a week. This was to catch

insects which emerge as adults immediately (i.e. *D. similis* and its parasitoid species). The inflorescences were kept moist by spraying the inflorescences with water twice a week. After emergence of all insects had stopped, we dissected ten random spikelets from each of ten random inflorescences (519 florets in total) following the methods of McKone et al., (2001) to check for overwintering live puparia of *D. similis* or its parasitoids.

5.2.5 Small emergence traps

As a part of a larger field experiment, in mid-February 2005, we counted and collected inflorescences of 128 *Chionochloa* plants in two different altitudes (68 plants from 450 m site and 60 plants from 1070 m site, hereafter refered to as the 2005 collection) and another 120 *Chionochloa* plants were counted and collected from the same two altitudes (60 plants from each site, hereafter refered to as the 2006 collection) in mid-February 2006. Every bunch of inflorescences from each individual plant was placed in a clay pot, which had a 1 mm polyester mesh bag placed inside it. Clay pots enabled the exchange of moisture between the contents of the pot and the surrounding soil to avoid desiccation of the insects and also to provide protection from excess moisture. A small amount of potting mix and then the inflorescences were placed into the mesh bag. The potting mix was sterilized before being placed inside the pots in order to prevent contamination by insects from other sources. The potting mix was added to provide a pupation substrate if any insects that leave the florets require this. The mesh bags were sealed by tying a knot and the pots then buried level with the soil surface so the bag was level with, or slightly above, the soil surface. That way, the insects were subjected to natural photoperiod, air temperature and humidity. At the 1070 m site pots were buried no more than 1 m away from the host plant which had provided the inflorescences, while at the 450 m site pots were buried in a sheep-proof area, about 20 m away from the host plants. Where necessary, small plastic ‘shade panels’ were placed to the north side of the pots to protect them from overheating in direct sun.

Pots from the 2005 collection were left undisturbed in the field until mid-October 2005. At that time, the mesh bags were opened and each pot was covered with an emergence trap. Emergence traps were made from pots of the same diameter as the clay pots fastened in place with wire pegs. Two clear plastic tubes were fixed to the side of each emergence trap each carrying a removable vial for collection of emerging insects. The transparent plastic 5 ml vials had open

ends covered with 1 mm mesh to allow air to circulate. Emerging insects were collected once a week from mid-October 2005 to early March 2006 in each of the two sites. Collected insects were killed in the freezer overnight, then identified to species, counted, and preserved in 70% ethanol.

At the end of the first season, when no more insects emerged from the pots, the emergence traps were removed and the mesh bags re-sealed to allow larvae to over-winter another season. In the second and third summers emerging adult insects were collected again using the same collecting methods from mid-October 2006 to early-March 2007 and from early-November 2007 to early-March 2008, respectively. The same procedure was done to the 2006 collection, where insects were collected from mid-October 2006 till early March 2007 and again from early November 2007 to early March 2008.

5.2.6 Data analysis

Calculating percentages of parasitism in insect populations can be problematic. Van Driesche (1983) recommends determining the rates of absolute parasitism in the host's larval stage after the parasitoids have finished ovipositing but before mortality from various factors occurs. However, this is not always possible. For example, *E. chionochloae* larvae enter prolonged diapause inside the florets of *Chionochloa* (Kolesik et al., 2007) and opening these florets to search for parasitism reduces the survival of the larvae (see Chapter 6). In other cases parasitoids cannot be reliably detected externally. Therefore we calculated percentages of parasitism using the adult stages of all species (hosts and parasitoids) for *M. calamogonus*, *E. chionochloae* and *D. similis.*

The parasitism rate for *M. calamogonus* could not be calculated because we did not know how many *Z. tortriciphaga* parasitize *M. calamogonus* (see below) and that prevented us from calculating the percentage of the other parasitoids. The parasitism rate for *D. similis* was calculated from the last bulk collection as the total number of adult parasitoids emerging divided by the number of adults of all species (*D. similis* plus parasitoids) emerging. Parasitism rates for *E. chionochloae* were calculated as the total number of parasitoid adults emerging for each

collection over several seasons (three and two years for the 2005 and 2006 collections respectively) from the small emergence traps, divided by the total number of adults emerging (*E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* species). A Pearson's Chi-square test with a Yates' correction was done to test whether parasitism rates of *Gastrancistrus* sp. and *Zelostemma chionochloae* differed across elevations. Tests were done separately for each year of emergence.

5.3 Results

In total we found seven different parasitoids, as summarised in Table 5.1.

Table 5.1. Parasitoids found attacking seed and flower predators of *Chionochloa* grasses. These include all the parasitoids reported previously in the literature, except for a possible Eulophid on *D. similis* mentioned by Cone (1995), see text.

Host insect (seed predator)	Parasitoid	Notes
Megacraspedus calamagonus (Lepidoptera: Gelechiidae)	*Zealachertus tortriciphaga* Berry (Hymenoptera: Chalcidoidea: Eulophidae)	The most common parasitoid of *M. calamogonus*. Apparently gregarious. This is a new host record, the wasp previously reported mainly on Tortricid moth larvae. Probably the eulophid mentioned by White (1975) and Cone (1995) [species 1 in the Introduction] but White thought its host was *D. similis*.
	Diadegma sp. (Hymenoptera: Ichneumonidae: Campopleginae)	An undescribed species. Uncommon. Mentioned by White (1975) [species 2 in the Introduction].
	Dolichogenidea sp. (Hymenoptera: Braconidae: Microgastrinae)	An undescribed species. Uncommon. May be the same taxon mentioned by Cone (1995) [species 3]
	Uclesiella sp., (Diptera: Tachininae: Voriinae)	An undescribed endemic fly. This is the first record of it, and only the second species reported in this endemic genus.
Diplotoxa similis (Diptera: Chloropidae)	*Callitula* sp. (Hymenoptera: Pteromalidae: Pteromalinae)	An undescribed species. This is apparently the first record of it.
Eucalyptodiplosis chionochloae (Diptera: Cecidomyiidae)	*Gastrancistrus* sp., (Hymenoptera: Pteromalidae: Pireninae)	Common. The species is undescribed. Mentioned by Sullivan (1993) and Cone (1995) [species 5] but they were unsure of the host.
	Zelostemma chionochloae Buhl, (Hymenoptera: Platygastridae: Platygastrinae)	Common, but only very recently reported and named by Buhl et al., (2008) [species 6].

5.3.1 *Megacraspedus calamogonus*

We found four different parasitoids from four different families (3 hymenopterans and one dipteran) which attack *M. calamogonus*:

1. *Zealachertus tortriciphaga* Berry (Hymenoptera: Chalcidoidea: Eulophidae). This is an idiobiont parasitoid (i.e., it prevents any further development of the host after initial parasitization), which emerged in the lab from late January to mid-February 2005. It was the most abundant parasitoid species from *M. calamogonus* and much smaller than the other two hymenopteran wasps parasitising this moth (Figure 5.2). The adult size was very small (<2 mm length) relative to the final size of its adult host (<6 mm length) and the large number of adults (n = 149) came out in waves. We therefore suggest this species is gregarious but we do not know how many *Z. tortriciphaga* parasitize *M. calamogonus* on average (to determine this would require rearing single *M. calamogonus* larvae separately so that the wasps emerging from each could be counted). Specimens are preserved in the New Zealand Arthropod Collection (NZAC), Landcare Research, Mt Albert.

Table 5.2. Percentage parasitism of *Chionochloa* seed predators, based on total numbers of adults emerging of the three seed predators and their parasitoids. Emergence of *E. chionochloae* and its parasitoids is recorded from two different collections, each from two different altitudes. Emergence was recorded over three seasons 2006-08 for the 2005 collection and 2007-08 for the 2006 collection; the percentage parasitism in each case is based on total emergence across all seasons. *M. calamogonus* and *D. similis* and their parasitoids all emerged within one year.

Species	Site	Collection	Number of adults emerged	% parasitism	Total parasitism
M. calamogonus	450 m	2004	23		*
Zealachertus		2004	149		
Diadegma		2004	2		
Dolicogeniea		2004	14		
Uclesiella		2004	13		
D. similis	1070 m	2008	1089		1.0
Calitulla		2008	11	1.0	
E. chionochloae	450 m	2005	3157		40.9
		2006	1965		44.8
	1070 m	2005	405		43.1
		2006	402		36.0
Gastrancistrus	450 m	2005	854	16.0	
		2006	299	8.4	
	1070 m	2005	240	33.7	
		2006	114	18.2	
Z. chionochloae	450 m	2005	1332	24.9	
		2006	1297	36.4	
	1070 m	2005	67	9.4	
		2006	112	17.8	

* could not be calculated, see text.

2. *Diadegma* sp. (Hymenoptera: Ichneumonidae: Campopleginae). This is an undescribed species (J. Berry, pers. comm.) with adults 6-7 mm long (See Table 5.2 for body volume). The koinobiont (i.e., delays the immediate killing of its host) wasps emerged in the lab in early February 2005. The cocoon of this species (4-5 mm) is brown with silky white 'strings' surrounding it (Figure 5.1B). Nothing else is known of its biology. We found only two individuals (a male and a female) of this wasp species. Specimens are preserved in NZAC.
3. *Dolichogenidea* sp. (Hymenoptera: Braconidae: Microgastrinae). This is probably an undescribed endemic species. Adults are 3-4 mm long (Figure 5.1C). These idiobiont wasps emerged in the lab from late Januray to early February 2005. Its cocoon is white with silky white 'strings'. Nothing else is known of its biology (J. Berry, pers. comm.). Specimens are preserved in NZAC.
4. *Uclesiella* sp., (Diptera: Tachininae: Voriinae). Specimens are preserved in NZAC. This undescribed species (John Dugdale, per. comm.) is dark brown and has a rather elongate shape and slender posterior spiracle stems (Figures 5.1D and 5.1E). The larvae feed on the host larvae but do not consume the cuticule. The remains of the host are pushed from the inside to both ends of the original larva, probably with other excrement produced by the developing larva. At this stage the legs of the host are still visible. The feeding and moving of the fly larva inside the host is easily noticeable and the host cuticle turns dark brown and persists outside the parasitoid's puparium (Figure 5.1F). This parasitoid is a koinobiont and the pupae over-wintered inside the cocoon and emerged in the lab in the following spring in mid October 2005. Specimens are preserved in NZAC.

Both *Zealachertus tortriciphaga* and *Dolichogenidea* sp. are idiobionts and kill their host immediately after they finish feeding, whereas *Diadegma* sp. and *Uclesiella* sp. are koinobiont. To summarize, we found 149 *Z. tortriciphaga*, which is probably a gregarious species, 2 *Diadegma*, 14 *Dolichogenidea* and 13 *Uclesiella*. Only 23 of the 73 cocoons produced *M. calamogonus* adults. Even without knowing the exact parasitism rates, it appears that *M. calamogonus* suffers high levels of parasitism.

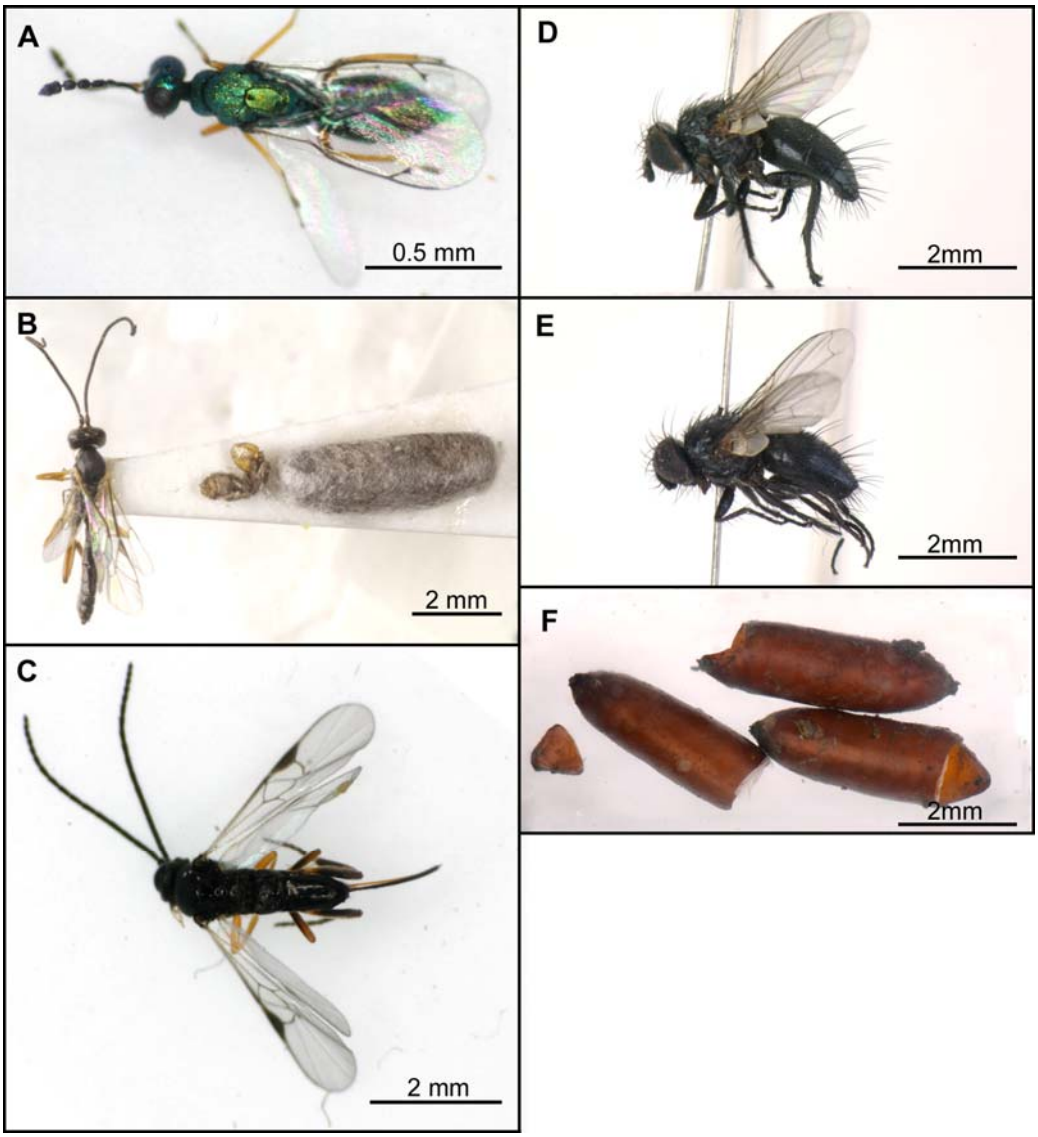

Figure 5.1. The parasitoids of *Megacraspedus calamogonus*: (A) *Zealachertus tortriciphaga* adult female (B) *Diadegma* sp. adult male and its puparium with *M. calamogonus* larval remains (C) *Dolichogenidea* sp. adult female (D, E, F) *Uclesiella* sp. adult female, adult male, and three empty puparia respectively. Photos (A) and (C) by MS, all others by Matt Walters.

5.3.2 *Diplotoxa similis*

From the last bulk collection, 1089 adult *D. similis* were found from late January to early March 2008, and 11 adult wasps from the genus *Callitula* (Hymenoptera: Pteromalidae: Pteromalinae; John La Salle, pers. comm.) emerged from late February to late March 2008. These wasps had sexual dimorphism where males had longer antennae than females. Although there were also *E.*

chionochloae larvae in the bulk collection, we assume the *Callitula* parasitises *D. similis* which emerges a bit earlier. The known parasitoids of *E. chionochloae* emerge in subsequent seasons, like the cecidomyiid itself. This suggests a parasitism rate of 1.0% of the initial number of *D. similis* larvae (Table 5.2). Dissections of florets after emergence found 46 empty puparia from *D. similis* and one *D. similis* puparium containing an unidentifiable dead wasp, but no evidence that any appreciable number of *Callitula* delay emergence to overwinter as a larva or a pupa. Specimens are preserved in NZAC.

5.3.3 *Eucalyptodiplosis chionochloae*

At least two parasitoids from two different families attack *E. chionochloae*:

1. *Gastrancistrus* sp. (Hymenoptera: Pteromalidae: Pireninae). We found *Gastrancistrus* adult females and males (Figures 5.2A and 5.2B respectively) for the first time in October 2005 in the lab from the second bulk collection of *Chionochloa pallens* from the 1070 m site at Mt Hutt. We also observed females ovipositing into florets of *Chionochloa pallens* containing *E. chionochloae* first instar larvae (Figure 5.2C) collected by D. Kelly at 1540 m on Mt. Hutt on 3 March 2007. Parasitized *E. chionochloae* larvae show a typical internal red dot (Figure 5.2D). *Gastrancistrus* sp is considered a specific koinobiont parasitoid of *E. chionochloae.* Adults were present at the 450 m site from December, which is just after all *E. chionochloae* adults have emerged (Figure 5.3) and around the time that the cecidomyiid eggs and first instar larvae are present (McKone et al., 2001). *Gastrancistrus* parasitised about double the percentage of larvae at 1070 m as at 450 m. There were significantly higher rates of parasitism at the higher site for both collections: 2005 ($\chi^2 = 77.44$, $P < 0.001$, $n = 4656$) and 2006 ($\chi^2 = 25.54$, $P < 0.001$, $n = 2780$) when emergence over three (2005 collection) or two (2006 collection) seasons were pooled (Table 5.2). The geographic distribution of this species is unknown but we assume it is likely to be similar to that of *E. chionochloae.* Specimens are preserved in NZAC.
2. *Zelostemma chionochloae* Buhl, (Hymenoptera: Platygastridae: Platygastrinae) (Figures 5.2E and 5.2F). This recently described species (Buhl et al., 2008) was first found in October 2005 from our second bulk collection, together with its host. Its biology is similar to that of *Gastrancistrus* and it is also considered a host-specific koinobiont

parasitoid of *E. chionochloae.* From our emergence traps placed in the field we found that *Z. chionochloae* parastisized more than twice the percentage of larvae at the 450 m as at the 1070 m site. There were significantly higher parasitism rates at the lower site for both the 2005 ($\chi^2 = 32.94$, $P < 0.001$, $n = 4761$) and the 2006 ($\chi^2 = 60354$, $P < 0.001$, $n = 3776$) collections (pooled emergence over three and two seasons respectively; Table 5.2), the opposite pattern to that shown by *Gastrancistrus*. The net effect was that *Z. chionochloae* was responsible for nearly three-quarters of all parasitism at the lower site, but only about one-third of all parasitism at the higher site. Emergence started in mid-November at the 450 m site, before the emergence of *E. chionochloae* (Figure 5.3 and see also Figure 5.12 in Buhl et al., (2008)). It is not known whether *Z. chionochloae* attacks the eggs and/or early instar larvae of *E. chionochloae*. Specimens are preserved in the Auckland Museum, New Zealand, AMNZ80716.

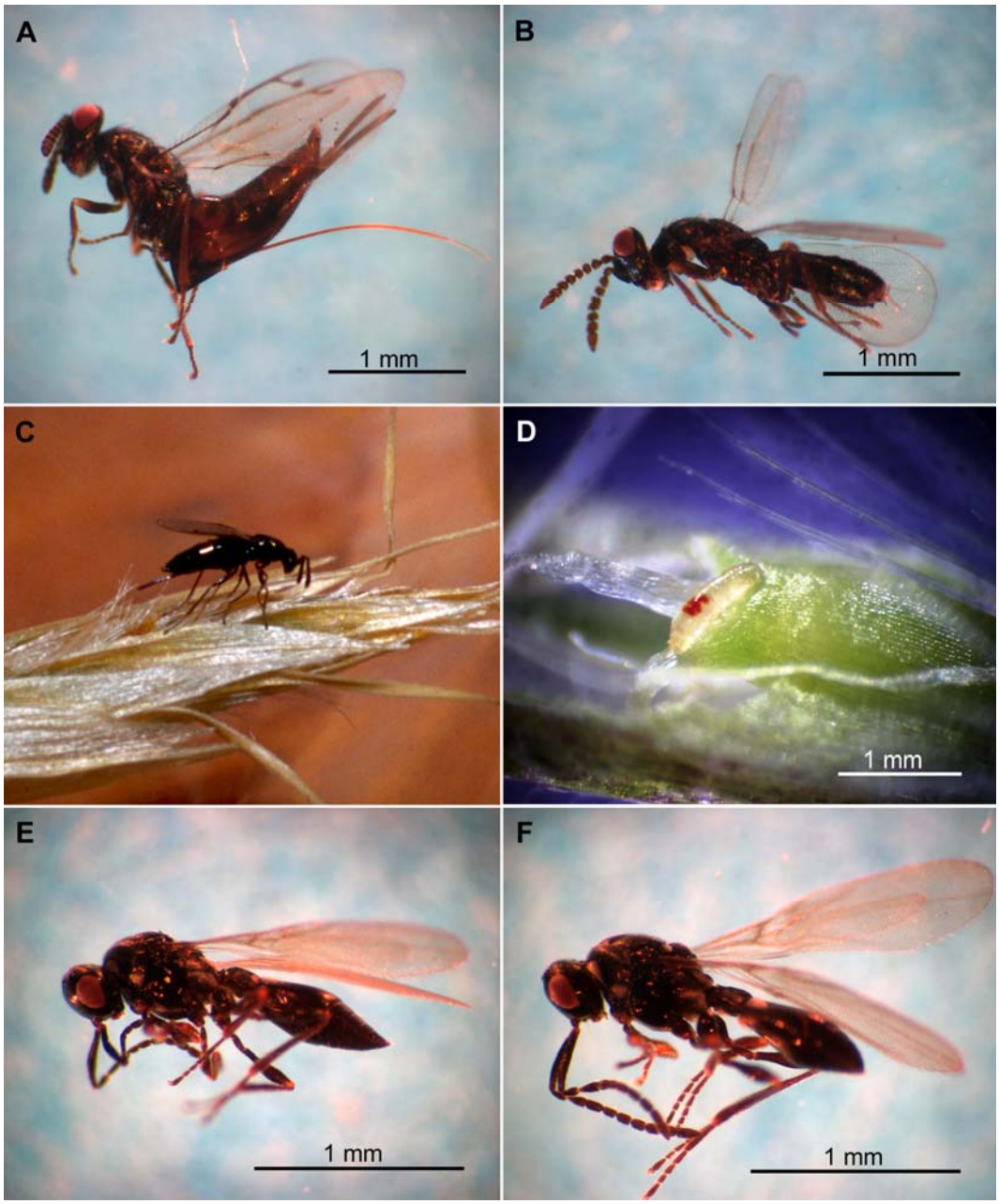

Figure 5. 2. The parasitoids of *Eucalyptodiplosis chionochloae*: (A) *Gastrancistrus* sp.adult female, (B) *Gastrancistrus* sp. adult male (C) *Gastrancistrus* sp. female ovipositing in floret of *C. pallens* collected from Mt Hutt 1540 m on 3 March 2007. (D) *E. chionochloae* first instar larva from the florets shown in (C) containing *Gastrancistrus* egg (red spot). The larva is on an ovary of *C. pallens.* (E) *Zelostemma chionochloae* adult female (F) *Zelostemma chionochloae* adult male. Photo (C) by DK all others by Jan McKenzie.

Over the three years, more than 10,000 adult *Eucalyptodiplosis chionochloae*, *Gastrancistrus* sp. and *Zelostemma chionochloae* were collected (Table 5.2). Parasitism levels on *E. chionochloae* by the two parasitoids were high. When all adults emerging over several years from each

collection were summed, parasitism rates calculated on these totals (Table 5.2), and then rates averaged across the four collection year x site combinations, the overall levels of parasitism were 41.2% (range 36.0 - 44.8 in different site/years), of which 19.1% was due to *Gastrancistrus* and 22.1% to *Z. chionochloae*.

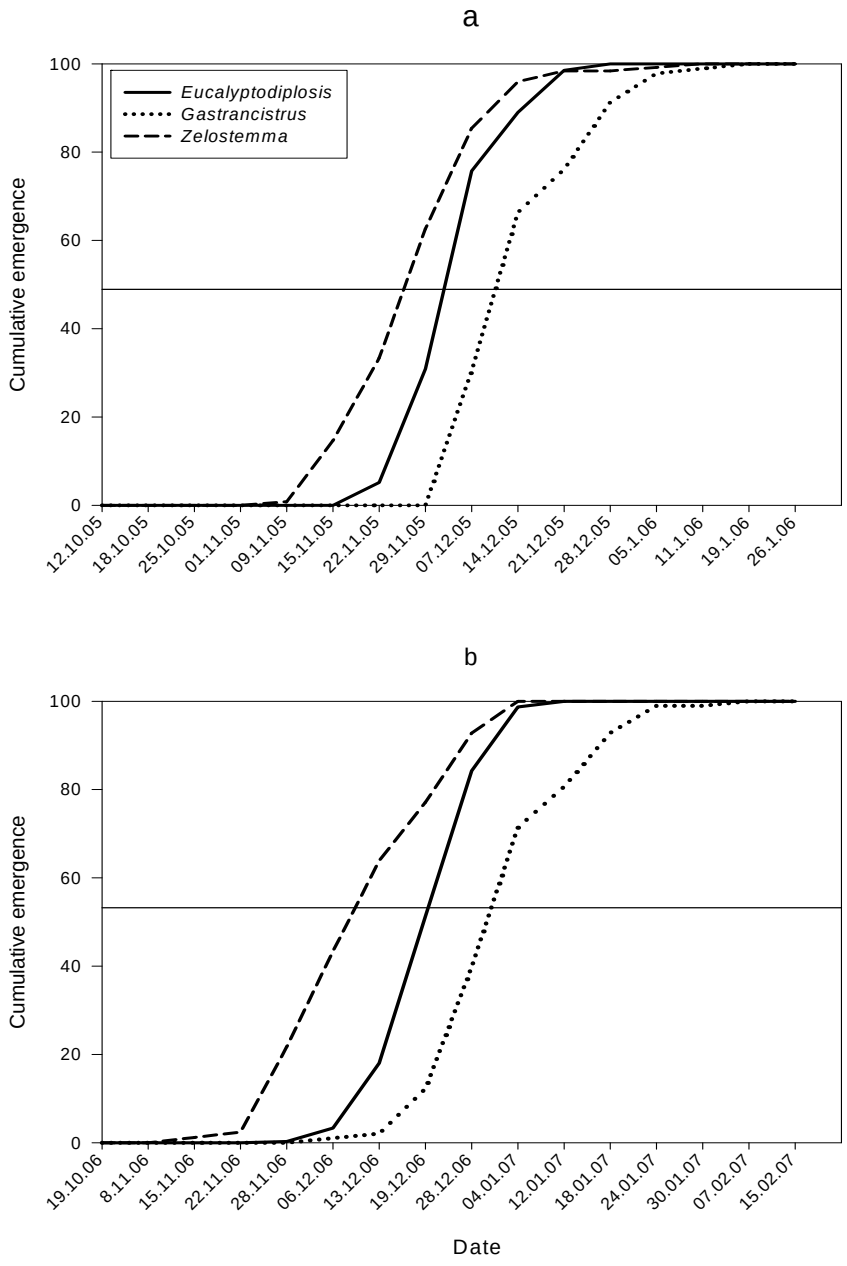

Figure 5.3. Cumulative within-season percent emergence of *E. chionochloae* and its parasitoids at the 450 m site over two seasons (a) 2005/06 and (b) 2006/07 shown as cumulative emergence graphs. Note that in the 2005/06 season most *Z. chionochloae* emerged before *E. chionochloae* but a small number emerged relatively late.

5.4 Discussion

It has taken many years to gain a clearer understanding of the parasitoids of *Chionochloa* seed predators. White (1975) reported the existence of two different wasps. Cone (1995) found one of White's and added three more. Here we give information on seven parasitoids. The three herbivorous insects vary in their parasitoid load, as summarised below.

5.4.1 The parasitoids of *M. calamogonus*

Zealachertus tortriciphaga was found to feed on *M. calamogonus*. This wasp is known to feed on moths in the family Tortricidae (hence its specific name) and therefore our data adds a new host record for this parasitoid, feeding on a different family of moths (Gelechiidae) (J. Berry, pers. comm.). Its known geographical distribution is from the North Island, South Island and Stewart Island (Berry, 1999). *Zealachertus tortriciphaga* has been reared from five species of Tortricidae (*Ctenopseustis obliquana*, *Planotortrix octo* or *excessana*; *Pyrgotis plagiatana,* and *Atamacta alopecana*), and also from one species in the Gracillariidae, *Caloptilia elaeas* (Berry, 1999). The first three listed tortricids are all known pests of horticulture and orchids in New Zealand (Suckling et al., 1998; Stevens et al., 2002; Wearing et al., 2003). This generalist wasp species may therefore feed on alternate hosts when *M. calamogonus* is uncommon or absent in low flowering years. We could not determine whether this species is gregarious or solitary, however other eulophids were found to be gregarious with more than one larva per host (Clausen 1962; Brockerhoff & Kenis 1996). More specifically, two other species of the same genus are recorded as being gregarious, *Zealachertus nothofagi* Boucek (Berry, 1999) and *Z. holderi* with 4-15 pupae found in each host (Holder, 1990). Jo Berry looked through the material examined in the *Zealachertus tortriciphaga* section (Berry, 1999) and stated that: "it appeared that on a number of occasions more than one wasp has been reared from a host" (J. Berry, pers. comm.). Additionally, the final adult size differed between individuals, from very small (≤ 1.5 mm) to relatively large (≥ 3.5 mm) (Cone, 1995; MS pers. observ.). Cone (1995) suggested that it may be due to sexual dimorphism: in gregarious wasps being a smaller male may be less of a disadvantage than being a small female (van den Assem et al., 1989). Alternatively, there may

sometimes be too many parasitoids in one host for the wasps to reach their maximum potential size.

The undescribed species of *Diadegma* wasp parasitising *M. calamogonus* we found is probably the same taxon as White's (1975) report of hymenopterous larvae. There are few other described species from this genus in New Zealand: three New Zealand native species, *D. novaezealandiae* (Azidah) (Azidah et al., 2000), *D. agens* and *D. mulleri* (Valentine & Walker, 1991) and two introduced species to New Zealand, probably from Europe, *D. fenestralis* and *D. semiclausum* (Holder, 1990; Valentine & Walker, 1991; Azidah et al., 2000). However, at least 50 more undescribed species are known to be present in New Zealand (J. Berry, pers. comm.) so this group is in need of major taxonomic revision to classify and name all the new taxa, including the one feeding on *M. calamogonus*.

We could find no information in the literature relevant to the *Dolichogenidea* sp. we found from *M. calamogonus.* However, another species in the same genus, *D. tasmanica*, is a known parasitoid of leafroller miners (Lepidoptera: Tortricidae) which in their larval stage are horticulture pests in New Zealand (Charles et al., 1996; Suckling et al., 1998; Burnip & Suckling, 2001). *D. tasmanica* emergence peaks in December and March and it parasitizes the first two instars of its host (Burnip & Suckling, 2001).

The tachinid flies from the New Zealand Voriini species are known to be associated with lepidopteran larvae (Dugdale, 1969). Malloch (1938) described the genus *Uclesiella*, with one wild-caught species*, U. irregularis* Malloch and placed it under the Voriini, which is a very distinct tribe that was earlier regarded as a subfamily by many authors. This is the first report of a second species in *Uclesiella*.

M. calamogonus has a high parasitoid load, in terms of both numbers of parasitoid species and percentages of larvae parasitised, which makes the interactions between the species more complex. However, at least one of these species (*Zealachertus*) may be gregarious which means parasitism levels may be lower as many parasitoid adults could emerge from one *M.*

calamogonus larva. Further study would be valuable in order to better understand the relationships between the different species in different trophic levels. It is noteworthy that *M. calamogonus* has a predominantly lower-altitude distribution, compared to *D. similis* and *E. chionochloae* (Sullivan & Kelly, 2000; McKone et al., 2001; Hay et al., 2008). In some cases overseas, parasitism is higher at lower altitudes (Hodkinson 2005 and references therein), so the high number of parasite species attacking *M. calamogonus* may be partly because of where it is found. However, in terms of percent of larvae parasitised, *E. chionochloae* also shows relatively high losses, despite having a more high-altitude distribution (Sullivan & Kelly 2000; McKone et al., 2001; Hay et al., 2008).

5.4.2 The parasitoids of *D. similis*

The *Callitula* species which probably parasitizes *D. similis* is reported here for the first time. Dissections of plant material after emergence suggest that *Callitula* sp. do not overwinter as pupae but emerge at the end of the summer season and presumably overwinter as adults, similar to adults of *Diplotoxa similis*. Cone (1995) found elophid wasps inside empty *D. similis* cocoons, however we failed to find these wasps. The pupae of the eulophid wasps found by Cone were described as small with only two antennal segments; however these may be abnormal insufficiently developed pupae as wasps always have more than two antennal segments. She found four adults of these wasps, of which one was outside a *D. similis* empty cocoon but inside the floret and therefore she considered that these wasps were definitely feeding on *D. similis*. According to a photo of the adult wasp presented in Cone (1995), the adult wasp is about 2 mm long. Unfortunately, Cone did not describe the adult wasps she found and the photo which she presented gives too little information to say whether our *Callitula* wasps are the same taxon as hers. Cone (1995) found live pupae, which did not show any sign of advanced development inside *D. similis* cocoons when most *D. similis* adults had already emerged. She therefore concluded that these wasps probably emerge later than the adult *D. similis*. Our *Callitula* wasps started their emergence after the last *D. similis* emerged, which matches Cone's description. Our adult *Calitulla* wasps were about 2 mm long, similar to the size of Cone's adult wasp. It may be that we found the same species Cone did, but her identification of the wasp's family was wrong.

Otherwise we have found a different species of parasitoid and *D. similis* may be parasitized by more than one species of wasp.

Perhaps the most remarkable feature of the parasitism of *D. similis* is the very low percentage of larvae attacked (1%). This is noteworthy given that *D. similis*, of all the three seed predators, shows least variation in numbers from year to year (McKone et al., 2001) and thus represents an easier "target" for a parasitoid. In other words, *D. similis* does not try to escape parasitism by unpredictable emergence from diapause (unlike *E. chionochloae*), yet it has much lower levels of parasitism than *E. chionochloae*.

5.4.3 The parasitoids of *Eucalyptodiplosis chionochloae*

Pireninae (Pteromalidae) parasitoids are known to develop in cecidomyiidae which create galls on various plants (Goulet & Huber, 1993). A Japanese species of *Gastrancistrus* is known to parasitise a cecidomyiid (*Asteralobia sasakii*) which makes galls in shoots of *Ilex* spp. (Tabuchi & Amano, 2003). In New Zealand, there are likely to be a number of *Gastrancistrus* species but not much is known about their biology or taxonomy (J. Berry, pers. comm.). A single male of a *Gastrancistrus* species was first found by Sullivan (1993) at Scotts Saddle, 1060 m elevation, Mt Hutt and later Cone (1995) found relatively large numbers of males and females in March 1995 at Mt Hutt, 1600 m elevation.

However, both Sullivan and Cone could not determine the host for these parasitoids. A single *Gastrancistrus* female was collected close to ovipositing *E. chionochloae* females in the Otira Valley (42º 53.8'S, 171º 32.6'E, 1000 m altitude) on 13 January 2000, however only in November 2005 was it identified as *Gastrancistrus* sp. (McKone et al., 2001 and DK and MS unpubl. data). This *Gastrancistrus* sp. can enter prolonged diapause inside its host for at least two years to synchronize with its host. We assume that it is a specific kiniobiont parasitoid of *E. chionochloae.*

Z. chionochloae biology and ecology is presented in detail in Buhl et al., (2008). Kolesik et al., (2007) were the first to report this parasitoid species. It is surprising it has not been reported

before, since it was very common in our emergence counts from all the bulk samples, although previous work (Sullivan 1993; Cone 1995) has concentrated on higher-altitude sites whereas we found *Z. chionochloae* to be more abundant at 450 m.

As shown in Figure 5.3, adult *Z. chionochloae* and *Gastrancistrus* sp. may be still present in the field after *E. chionochloae* adults can no longer be found. *E. chionochloae* parasitoid wasps need to oviposit as many eggs as they can in order to maximize their fitness. Because the *E. chionochloae* season is rather short (adults are present in the field for only about 4 weeks, which means that they must lay their eggs within that time) and because *Z. chionochloae* and *Gastrancistrus* sp. oviposit their eggs in their host's eggs and/or 1st instar larvae (yet to be ascertained), they will maximize their fitness if the population lifespan will be longer than that of their hosts.

Adult *Z. chionochloae* emerge earlier than *E. chionochloae* while adult *Gastrancistrus* sp. emerge only later, during and after peak abundance of *E. chionochloae* adults (Figure 5.3). Adults of both wasp species can be found in the field after no more adult *E. chionochloae* are present. The two species of parasitoids probably experience inter-specific competition for food resources. We believe that both parasitoid insects are not gregarious but solitary as the adult final size is only slightly smaller than the size of the adult host. In many solitary species, only one parasitoid individual can complete development in its host (Mackauer, 1990; Godfray, 1994). If other larvae are present in the same host, they will be eliminated by either physiological suppression (e.g. toxic secretion, asphyxiation or starvation) or physical fight (Mackauer, 1990).

There are three possible scenarios for the interactions between *Z. chionochloae* and *Gastrancistrus* sp.: (a) *Multi-parasitism* – occurs when a female parasitoid of one species lays an egg inside a host, which was already parasitized by another species of parasitoid. In that case, larval competition for host resources will lead to decline in offspring survival (Mackauer, 1990; Godfray, 1994). This strategy will be most advantageous when hosts are scarce and eggs of the parasitoids are unlimited (Bakker et al., 1985); (b) *Interspecific* (or *Hetero-specific*) *host discrimination* – some parasitoid species can detect that a host has been parasitized by another

species and in that case multi-parasitism will frequently be avoided (Turlings et al., 1985; Mackauer, 1990; Pijls et al., 1995); (c) *Heterospecific ovicide*: occurs when the second female kills the egg of a previous parasitoid either by eating or stabbing it to eliminate competition with her offspring (Mackauer, 1990; Collier et al., 2007). Because adult *Z. chionochloae* emerge earlier than *Gastrancistrus* sp., their eggs might be present in the hosts earlier than those of *Gastrancistrus*. This would be advantageous for *Z. chionochloae* if *Gastrancistrus* females have inter-specific host discrimination abilities which prevent them from laying their eggs in the already parasitized host, or if the larvae of *Z. chionochloae* are better fighters than larvae of *Gastrancistrus* in case of multiparasitism.

However, a later oviposition can be advantageous to the offspring of *Gastrancistrus* if their females can eliminate previous eggs of a different species (ovicide) or if *Gastrancistrus* larvae are better fighters than *Z. chionochloae* (which might be difficult if the *Z. chionochloae* larvae have already begun to develop and are larger, although both wasps and the host have extended diapause so early-laid wasp eggs may not start to develop into larvae right away). Host discrimination or ovicide could be expensive for *Gastrancistrus* in terms of time and resources spent in searching for the right host (host discrimination) or the time cost to the female to eliminate previous eggs (ovicide), which would have to be balanced against the cost of finding the next unparasitized larva. Observations of *Gastrancistrus* females searching *Chionochloa* florets suggests that probing a floret frequently takes a long time (several minutes; D. Kelly, pers. obs.), but that time may be spent in finding larvae and the right organ for oviposition within the larva rather than time to investigate the larva, or kill the egg of a previous parasitoid. However, if *Gastrancistrus* larvae are bad fighters or the number of hosts is limited and females cannot eliminate previous eggs, then *Gastrancistrus* may discriminate previously parasitized hosts and choose to oviposit in other unparasitized hosts.

Although *Z. chionochloae* adults are present earlier than *Gastrancistrus* adults, female *Z. chionochloae* might be able to lay eggs later on in the season, after *Gastrancistrus* oviposition. In that case, *Z. chionochloae* may be the second larvae in the same host. At the 450 m site more adult *Z. chionochloae* were found in the first emergence year (2005/06) than those of

Gastrancistrus sp. however, during the second emergence year (2006/07) fewer *Z. chionochloae* than *Gastrancistrus* emerged. That might suggest that *Gastrancistrus* sp. better survive prolonged diapause. *Gastrancistrus* insects are usually larger than *Z. chionochloae* (Figure 5.2A and 2B vs. 2E and 2F) and may have more resources such as dry fat and energy resources which enable them to survive prolonged diapause better.

Perhaps the most remarkable aspect of parasitism in *E. chionochloae* is the high levels of larvae lost: an overall mean of 41% across two sites each measured in two years. The losses were fairly evenly split between *Gastrancistrus* and *Z. chionochloae* (although that average hides the fact that *Gastrancistrus* was consistently predominant at the upper site while *Z. chionochloae* was predominant at the lower site).

To conclude, there are at least two species of host-specific parasitoids feeding on *E. chionochloae*, and both can enter prolonged diapause. *M. calamogonus* has four known parasitoids, of which three are hymenopteran and one is a dipteran species. *D. similis* apparently has at least one species of hymenopteran parasitoid but more study is required on this species. Parasitism rates are very high in *E. chionochloae*, but very low in *D. similis*. Parasitism rates could not be calculated for *M. calamogonus*, although they appear to be high as many parasitoid adults emerged relative to the number of *M. calamogonus* adults (but note that at least one of the parasitoids is thought to be gregarious). Parasitism rates seem high enough to possibly reduce local densities of *E. chionochloae* and maybe of *M. calamogonus*, which in turn would reduce seed predation in *Chionochloa*, and may alter the selective forces favoring mast seeding in the plant genus. However, it must be borne in mind that the reported high levels of seed predation by the three seed predators (White 1975, Kelly et al., 1992, Kelly & Sullivan 1997, Sullivan & Kelly 2000, Kelly et al., in press) are all derived from natural populations with unfettered access by the parasitoids. It may be that *Chionochloa* simply supports high densities of both the seed predators and their parasitoids – in other words both parasitoids and seed predators may be food-limited (bottom-up regulation), rather than the seed predators being predator-limited (top-down).

6. Prolonged predictive diapause of *E. chionochloae* and its parasitoids *Gastrancistrus* sp. and *Z. chionochloae*

6.1 Introduction

Many insects are capable of perceiving environmental cues such as photoperiod and temperature and respond accordingly with specific physiological, behavioural or morphological modifications that enable them to survive unfavourable conditions in a state of reduced metabolism i.e. diapause (Tauber et al., 1986; Danks, 1987; Saunders et al., 2002). Prolonged diapause is diapause for 12 months or longer where the insects miss one breeding season or more in order to increase their chances of survival (Hanski, 1988). Prolonged diapause may be associated with local adaptation to multi-annual variability among years in availability of resources (Tauber et al., 1986). Insects in prolonged diapause attempt to increase their offspring's fitness by reproducing in a better season with adequate resources (Hanski, 1988). Prolonged diapause usually occurs in univoltine insects where individuals from one cohort in prolonged diapause are synchronized with other individuals from different cohorts either in normal diapause or in prolonged diapause (Powell, 1989). An example of an insect with prolonged diapause is *E. chionochloae* (Kolesik et al., 2007).

Mast seeding by *Chionochloa* species, the host plants of *E. chionochloae*, is among the most extreme masting worldwide (Kelly et al., 2000). This most probably has evolved in order to satiate seed predators in high flowering years, and keep seed predator populations small in low flowering years (Kelly et al., 1992; Kelly, 1994; Kelly & Sullivan, 1997; Kelly et al., 2000; McKone et al., 2001; Kelly et al.). There are three known seed and flower predators to *Chionochloa*: *Megacraspedus calamogonus* (Lepidoptera: Gelechiidae); *Diplotoxa similis* (Diptera: Chloropidae) and *E. chionochloae* (Diptera: Cecidomyiidae) (McKone et al., 2001; Kolesik et al., 2007). *E. chionochloae* have the ability to enter prolonged diapause and emerge in high flowering years (Kolesik et al., 2007), which makes them a very sophisticated predator that is hard to satiate.

Prolonged diapause experienced by *E. chionochloae* may be either a risk-spreading (bet-hedging) or a predictive prolonged diapause. Risk-spreading diapause is a common behaviour among insects and plants that experience fluctuations in their environment (Menu, 1993b, 1993a; Menu et al., 2000). It is defined as '*breeding* [by a] *female* [which] *produces a mixture of offspring with different lengths of diapause; one expects that the mixture evolve to a value that maximizes the fitness of the breeding female'* (Hanski, 1989). Although Hopper (1999) in his review stressed that there is no clear evidence of bet-hedging, other studies did show the existence of this strategy in insects which experience prolonged diapause (Menu, 1993b; Menu & Debouzie, 1993; Danforth, 1999).

Hanski (1989) first defined the term 'predictive diapause' as '*insects* [which] *tend to emerge in years when the cone* [or seed] *crop is largest*'. This diapause is variable, with more insects emerging in years that are more favourable. The insects in diapause have the ability to detect environmental and plant cues and emerge accordingly (Hanski, 1989; Roques, 1989).

The difference between these two diapause types is the target individual whose fitness is affected by the diapause. Seger and Brockmann (1987) suggest that risk-spreading may be a disadvantage to the individual in diapause as the conditions at the time of its emergence may not be suitable and may therefore reduce their own fitness. According to Hanski (1989) in risk-spreading diapause the breeding female is affected by the diapause of her offspring while in predictive diapause the fitness of the diapausing individual itself is affected, although it is also in the breeding mother's interest that her offspring will make a good assessment of the environment and emerge accordingly (Hanski, 1989). In both strategies, there is a trade-off between diapause (a chance to increase growth rate and fitness of populations and individuals respectively) and survival. Such trade-offs may be the risk of mortality from predation (Menu & Debouzie, 1993; Menu et al., 2000), harsh environment (e.g. drought) (Menu, 1993b), disease (e.g. fungus attack) (Menu & Desouhant, 2002), loss of reproductive opportunities and slower rates of increase from delayed reproduction (Hanski, 1988). In addition, in risk-spreading diapause, the emerging offspring may have to face unsuitable environmental conditions as well, which will reduce their fitness. In contrast, in a predictive diapause, emerging insects can predict the season in which

they emerge according to some external cues. If prediction is done according to the right signals, this strategy can increase the diapausing insect's fitness and more offspring can emerge at the first real opportunity to have sufficient food, thus reducing the cost of their prolonged diapause. A number of studies demonstrated predictive diapause in insects (Claret & Carton, 1980; Annila, 1981; Hedlin et al., 1982; Brodeur & McNeil, 1989; Brockerhoff & Kenis, 1997; Garcia et al., 2002), however none demonstrated that by having predictive diapause the benefits are higher than the costs (i.e. the mean delay before reproducing in predictive diapause is minimized compared with risk-spreading diapause). Therefore I assume that the costs of prolonged diapause in both strategies are similar; however, the payoff from predictive diapause is greater than from risk-spreading diapause.

Several studies found predictive diapause in seed and cone predator insects. Bakke (1963) found that temporal fluctuation in cone production controls diapause of spruce-cone insects and their parasitoids in Norway. Powell (1989) studied the yucca moth *Prodoxus Y-inversus*. He established that these moths can stay in diapause for more than 16 years, terminating their diapause in response to environmental cues, such as warm winter temperatures. Roques (1989) suggested that not only environmental cues but also plant chemicals such as elevated nitrogen and gibberellic acids while the insects are still in direct contact with the plant, can control diapause. If insects using predictive diapause need cues to adjust their diapause, plant manipulations can be done to test that hypothesis. Indeed some of these suggested cues were previously tested, mostly from the plant's point of view. For example, Went (1953) suggested that temperature is a key factor in inducing flowering, and that each plant species has an optimum temperature at which it grows best. Therefore, altitude is most important in plant growth and stem elongation: in higher and colder temperatures a plant will grow more slowly than in lower, warmer altitudes. More specifically, Mark (1965c), Kelly & Sullivan (Kelly & Sullivan, 1997); Kelly et al., (2000) and Kelly et al., (2008) showed that heavy flowering by *Chionochloa* spp. occurred in the year after a warm summer (i.e., high mean temperature of previous January – 7th of February). Recently, Kelly et al., (2008) studied temperature cues in *Chionochloa* plants and found also same-season effect of temperatures between October and December on flowering intensity (inflorescences per tussock) of December to February of the

same season. Therefore, temperature cues, which are important for the synchrony of flowering in populations of *Chionochloa* species, should be detected by the insect predators if they have a predictive diapause.

Another factor affecting the elongation of plant tissues and therefore flowering intensity in plants occurs when there is an increase in Gibberellic Acid (Pharis & King, 1985). Gibberellin A_3 specifically is a native hormone to *Chionochloa* (Martin et al., 1993) and is known to induce flowering in long day photoperiods in other grass species (*Lolium temulentum*) (Evans, 1999; King et al., 2006) and in *Bryophyllum* spp. (Pharis & King, 1985). To my best knowledge, plant chemical signals controlling insect diapause were not studied using gibberellin A_3 (GA_3 hereafter) before, however it was done using gibberellin $A_{4/7}$ (Brockerhoff & Ho, 1997).

If gibberellin levels increase with elongation of plant tissue, and if relatively high temperatures encourage plant growth, then a combination of warmer temperatures and higher levels of gibberellins is predicted to have synergistic effects. Hiller et al., (1979) have studied GAs in carrots in relation to temperature and found seed-stalk height correlated with high temperature and applied GA_3. Pinthus et al., (1989) studied wheat species and found that genotypes which are responsive to temperature are also responsive to applied GA_3. During stem elongation of *Dendranthema grandiflorum*, higher levels of GAs were identified during the day (warmer temperatures) than at night (cooler temperatures) (Nishijima et al., 1997). A recent study on sunflower seedlings examined temperature and red/far-red light together with GA_3. Hypocotyl length was greatest when a combination of high temperatures and GA_3 were applied (Kurepin, Pharis and Reid, paper submitted). Conversely, other studies showed no relationship between GA levels and temperatures (Myster et al., 1997a; Myster et al., 1997b).

Two other known factors that increase endogenous gibberellin levels and increase flowering in plants are water stress, created for example by root pruning (Ross et al., 1985) and a synergistic effect which is created by a combination of root pruning and gibberellins. Such synergistic effects on plant growth and development were previously found in other plant species, such as Douglas-fir (*Pseudotsuga menziesii*) (Ross et al., 1985; Webber et al., 1985; Ross, 1991).

However, rainfall as a flowering cue may not be very accurate as it shows spatial heterogeneity (Norton & Kelly, 1988).

Host cues may play an important role in diapause induction and development of seed and flower eaters and their parasitoids (Askew, 1971; Beckage, 1985; Lawrence, 1986). However, if the parasitoids are exposed to a stochastic environment, they may use other external cues to terminate diapause (Askew, 1971; Doutt et al., 1976; Tauber et al., 1986). In addition, endophagous parasitoids are exposed to the physiological conditions within their insect host and would be expected to synchronize their own life cycles with those of their host, in particular the diapause stage (Tauber et al., 1986). This means that any parasite or predator of *E. chionochloae* should follow this behaviour to exploit its resources properly. Ringel et al., (1998) suggested that host diapause may serve as a refuge from parasitism. If the parasitoids do not have a prolonged diapause, the host may reduce parasitism by emerging and reproducing in a stochastic unpredictive way. On the other hand, if the parasitoids do have prolonged (risk-spreading or predictive) diapause, hosts will not be killed during the early developmental stages (Corley et al., 2004) and parasitoids will adopt host diapause-termination behaviour to control their emergence. In that case, predation levels on the host will be higher and parasitoids will play an important role in controlling densities of their hosts. *E. chionochloae* is parasitized by two specific parasitoid species, *Gastrancistrus* sp. and *Z. chionochloae*, which were both also found to enter prolonged diapause (Kolesik et al., 2007; Buhl et al., 2008). My goal in this study was to find out whether the insects from all three species use predictive diapause and if they do, whether they use external cues to emerge from prolonged diapause. Such cues can be environmental, relate to plant physiology (Roques, 1989), or a combination of the two. Both environmental and physiological cues were tested over two *Chionochloae* species from two elevations over three years of insect emergence.

Recently, Turnbull et al., (in prep.) studied the response of *Chionochloa* flowering to plant and environmental cues in order to detect the trigger used by the plants to synchronize their flowering between and within species. The full report to date (September 2008) of their results is presented in Appendix 1. Although they did not find a previous-year effect for the Warming

treatment, a current-year effect of treatments on flowering intensity was found (see also Kelly et al., 2008). Other current and previous year effects were found (i.e., gibberellin treatment). I was interested in finding out whether the insect seed predators were using the same cues to control their emergence/diapause as the plants to control their flowering/not. To do so, I compared the effects of manipulations done to plants in a previous year on flowering intensity the following year (Turnbull et al., in prep) to my data of insect emergence/diapause. Only effect of previous season were compared because the insect species have obligatory diapause of one winter so it is assumed they detect a cue according to which they decide whether to emerge next season or not while in their larval stage (either still feeding or diapausing).

I also aim to answer the following questions:

1. Do all three insect species use predictive diapause?
2. If they do use predictive diapause, do they use environmental cues, plant physiology cues or combinations of these environmental and plant physiology cues to control diapause and emergence?
3. Do the parasitoids use the same cues as their host or have they evolved to use other cues to synchronize with their hosts?

6.2 Methods

6.2.1 Study sites

The study area is located on Mount Hutt in Canterbury, New Zealand, on the eastern edge of the central Southern Alps, approximately 110 km west of Christchurch. Five different sites at three different elevations were studied: **450 m site:** located at the bottom of the mountain (43° 33.93' S, 171° 33.26' E), with *Chionochloa rubra* surrounded by exotic grasses. This site is a privately owned farm paddock and is exposed to sheep grazing a few times a year (Buhl et al., 2008). **1070 m site**: located half way up the skifield road (43°32.04´S, 171°32.97´E) dominated by 94% *C. pallens* and 6% *C. macra* (McKone, 1990; Kelly & Sullivan, 1997). **1300 m site:** at 43°31.15´S, 171°32.61´E with *C. macra* in addition to other native plants, such as *Aciphylla aurea* and

Celmisia spectabilis. This site is facing southeast and is highly exposed to wind (Kolesik et al., 2007). For a map of the area see Hay et al., (2008). For the differences between the three *Chionochloae* species see Table 1.1.

6.2.2 Plant Manipulations

The main goal of this experiment was to discover whether insects are using predictive prolonged diapause and if they do which cues they use in order to adjust their diapause. Plants were manipulated in two consecutive years (2004/05 and 2005/06 summer seasons) with the following treatments:

1. **Untreated plants – control group (C).**
2. **Plants treated with warmer temperatures (W).** Transparent open-topped plastic tubes (cloches) were placed on top of the plants from early December to March/April; the mean elevated temperature of *Chionochloa pallens* (measured in the tussock base) was 0.58°C with smaller increased (daily minima) of 0.36°C and higher increase (daily maxima) of 2.87°C (Kelly et al., 2008). The plants were subjected to natural photoperiod and humidity.
3. **Plants subjected to water stress by root pruning (P).** Roots of treated *Chionochloa* plants were pruned in early January and late December (2004/05 and 2005/06 respectively) using a shovel, which was inserted into the soil around half of the plant base at a 45° angle to the centre of the base of the plant.
4. **Plants treated with plant hormone gibberellic acid GA_3 (G).** Plants were treated with GA_3 (obtained from Professor Zhou Xie, Nanjing Agricultural University, China) at 150 ppm in an aqueous solution containing 0.1% surfactant (LI-700, Loveland Industries). Plants were sprayed to drip-off two times in the 2004/05 summer season (early and late January 2005). However, in the 2005/06 summer season plants were treated with GA_3 only once (late December 2005). In both years spraying was done using a plastic tube to prevent overspray of gibberellins onto other plants.
5. **Plants treated with both warm temperatures and GA_3 (WG).**
6. **Plants treated with both root pruning and GA_3 (PG).**

These treatments were a mixture of environmental cues (W to simulate warmer temperature

during summer and P to simulate drought), plant cues (GA_3 is a plant hormone that induces flowering) and combinations of environmental and plant cues (WG and PG). I tried to make the treated plants flower at a higher rate than the control group, and find out whether the insects respond to any of these treatments in terms of higher emergence and lower prolonged diapause.

6.2.2.1 Experimental design

The treatments described above were conducted at three elevations (450 m, 1070 m and 1300 m) in 2004/05. Ten replicate plants were chosen for each of the six combinations of treatments (60 plants in total) at each of the two lower altitudes (450 m and 1070 m sites) while only 30 individual plants were chosen at the 1300 m site with C, W and P treatments only (the G, WG and PG treatments were not applied at the 1300 m site because of a shortage of GA_3). The sites used tussocks selected by distance along four straight lines. Each line had many tussocks and every couple of metres a different treatment was applied to the nearest tussock in a fixed order so that treatments were interspersed.

In early February 2005, inflorescences of treated plants in all three elevations were counted (60 plants in each of the 450 m and the 1070 m sites and 30 at the 1300 m site). At the 450 m site all plants had inflorescences (and therefore flowers with seeds as food source for the insects), however at the 1070 m site, twelve plants out of sixty had zero inflorescences and at the 1300 m site seven out of thirty plants had zero inflorescences, reducing the number of replicates for subsequent insect rearing.

In December 2006, the same six regular treatments (C, W, P, G, WG, PG) were applied to ten plants in each of the 450 m and 1070 m sites, but to allow for multi-year treatment effects these (except the controls) were randomly divided into two groups of five. Five random replicates of each treatment were reapplied to plants given the same treatment in the previous year (5 X 5 = 25 plants), while another five new plants (untreated in the previous year) were added and treated. In addition, in order to have another sample from the 2005/06 flowering season at higher elevations, we counted inflorescences from six new control (not manipulated) plants at the 1300 m site for control groups.

6.2.2.2 Collection

Insects were allowed to oviposit and feed in the inflorescences naturally. In Late February 2005 inflorescences from treatment plants were collected from the 450 m and 1070 m sites with the exception of one plant from the 450 m site that was accidentally overlooked and therefore not collected. At the 1300 m site, only plants with more than three inflorescences were collected, which made a total of four control (C) plants and four pruning (P) plants. This collection is called the '2005 collection' throughout this paper. Every bunch of inflorescences from each individual plant was placed in a clay pot, which had a 1 mm polyester mesh bag attached to it from the inside. Clay pots enabled the exchange of humidity between the contents of the pot and the surrounding soil to avoid desiccation of the insects and to provide protection from excess moisture. A small sterilized amount of potting mix composed of sifted bark, peat and vermiculite, in equal ratios was inserted and then the inflorescences. The potting mix was inserted to keep insects that leave their florets alive. Pots were buried level with the soil surface so the inflorescence bag was level with or slightly above the soil surface. That way, all the insects were equally subjected to photoperiod, ambient air and humidity. At the 1070 m and 1300 m sites pots were buried no more than 1 meter away from their host plant while at the 450 m site pots were buried in a sheep-proof area, about 20 m away from the host plants.

In late January 2006 inflorescences of all the old and new treated plants were counted at all three sites, with the exception of 30 plants at the 1300 m site, which were not counted (see chapter 4, section 4.2.2 for details). These included 60 old + 25 new = 85 plants at 450 m; 60 old + 25 new = 85 plants at 1070 m; 6 new at 1300 m (176 plants in total). In February 2006, inflorescences of all the new treated and control plants from all three sites were cut off and placed in new clay pots with the same design as the ones in the previous year. This collection is named the '2006 collection' throughout this paper.

In October 2005 and October 2006, all the pots of both collections from all three sites were covered by emergence traps, which were placed on top of the clay pot (see Buhl et al., (2008)), whereas in November 2007 emergence traps of both collections were placed only on pots from the 450 m and the 1070 m sites.

Adult emerging insects were collected from all three sites once a week from mid October to late February (2005/06 summer season); mid October to early March (2006/07 summer season) and early-mid November to early March (2007/08 summer season). Insects were killed, identified and stored in 70% ethanol. In March 2007, the emergence traps were removed; mesh bags of clay pots resealed and insects left to over-winter for another season.

6.2.2.3 Dissections

When emergence of insects from the 2005 collection finished in autumn 2006, half the pots in each treatment at the 450 m, 1070 m and 1300 m sites were randomly selected and taken to the lab for dissection of inflorescences to count the number of *E. chionochloae* larvae still in prolonged diapause. These dissections are not the same dissections described in Chapter 4, which were done to random inflorescences collected in 2004/05 – 2006/07 flowering seasons in order to find pre-adult life stages phenology and predation levels by the three seed predators. All material in each pot from the 1070 m and 1300 m sites was dissected, whereas the material in each pot from the 450 m site was divided in half and only one half was dissected. The other half of the material from the 450 m pots was returned to the clay pot in the field. That was done because there was abundant material in the pots at the 450 m site. Pots were regularly watered in the lab to keep plant material and insects moist. *E. chionochloae* larvae found in each pot were counted and placed live in a new clay pot with a mesh bag and soil. All pots with live larvae (see below) were placed back in the field for another emerging season. These new pots were named with the same code as the original pot with the addition of the letter 'B' to indicate this plant material had been dissected.

Some of the larvae were accidentally stabbed during dissection and died, and these animals were not counted in the number of live larvae which were recorded as placed back into the field. Dissections also apparently did damage to the insects from two other causes. First, many insects suffered from the dry conditions at the lab and although they were watered regularly, many got dry, shrank and died. Second, insects that survived dissections were removed from their floret and placed in the soil without the floret's protection, which may have decreased their survival.

In agreement with Kolesik et al., (2007), *E. chionochloae* larvae found in the florets were mostly 3rd instar larvae. The number of *E. chionochloae* larvae per half-pot which were observed during dissections in March 2006 was compared to the counts of total adult insect emergence in the other (non-dissected) half-pot from *E. chionochloae* alone or from all three species (*E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* spp.) in the following summer. If dissections were a good predictor for the number of larvae still in diapause in each pot, I would expect the number of adults emerging from the other half-pot which was not dissected to be similar to the number of diapausing larvae I found in the dissections. To see if dissections systematically found fewer insect larvae than later emerged as adults, I ran a paired t-test to compare the number of *E. chionochloae* larvae that were found in the half dissected pots to the number of total adult insect emergence (*E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* spp.) from the half non-dissected pots for the 450 m. In all treatments, the number of emerging adults was larger than that of larvae found in the dissections. Nearly four times as many insects emerged in 2006/07 summer season from undisturbed half-pots (mean = 30.10) as were found in total as larvae in the dissected half-pots (mean =7.75), suggesting that the dissections were missing many larvae (d.f. = 27, t = -3.47, P = 0.001). I did not run this test for the 1070 m site because for the 1070 m data, entire pots rather than half-pots, were dissected so it would not have been possible to run the paired analysis used at 450 m. Also the numbers at the 450 m site were much higher and proved that many larvae were overlooked in the dissections and that the number of larvae found in the dissections was not reliable as an indicator for the number of larvae still in diapause in each pot.

I ran another paired t-test for both the 450 m and 1070 m sites to compare the number of diapausing *E. chionochloae* larvae that were found (in half dissected pots for 450 m site and in whole dissected pots for 1070 m site) to adult insect emergence (*E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* spp.) the following summer from the same dissected half (450 m site) or whole (1070 m site) pots when placed back into the field. This was done in order to check whether the dissections physically damaged the insects and their emergence rates. In this analysis I did not use pots with zero larvae found in dissections as no live larvae could emerge from these pots (they were not placed back into the field). Therefore there are two different means of larvae in the same half dissected pots when comparing to half non dissected pot and half dissected pot

(450 m site). Significantly more *E. chionochloae* larvae were found in the half pots (mean = 33.26, 450 m site) and whole dissected pots (mean = 44.51, 1070 m site) during dissections than the number of adults that emerged in the following season from these half (mean = 4.93) or whole (mean = 16.08) dissected pots (450 m and 1070 m respectively), in contrast to the previous analysis where more adults emerged ($t = 2.82$, d.f. = 19, $P = 0.01$ (450 m site); $t = 4.42$, d.f. = 22, $P < 0.001$ (1070 m site)). It might be that the reason for the low level of emergence was because larvae were still in diapause. However, this would require larvae to be more likely to stay in diapause after dissection than if undisturbed. It is more likely that dissections of florets damaged the insects, as at the emergence of adults from the non-dissected half pots in 2006/07 summer season was much higher.

I therefore decided not to dissect plant material at the end of 2006/07 as this did not give a reliable estimate of the number of diapausing insects. In analysis of total emergence from the pots at the 450 m site, for seasons after the dissections had been done, I used the actual field emergence from the undissected half multiplied by two to allow for half the material having been removed and dissected. The data from the dissected half-pots at this site were not used. At the 1070 m site, all material in a dissected pot was dissected so I had to use the field emergence of dissected material after it was placed back into the field. In this case, in the analysis an additional factor 'dissected/not' was included to allow statistically for the lower rates of emergence from pots after the material was dissected.

6.2.3 Data Analysis

1. Generalized Linear Models (GLMs) were used for the 450 m site, 2005 collection, to test whether emergence was affected by the five different treatments. At that site, emergence numbers in 2007 were corrected for pots which were dissected (i.e. in dissected pots, the number of insects emerging in 2007 from the undissected material was multiplied by two because half the material was removed for dissection in mid 2006) whereas that was not done to the non-dissected pots in this site. As for the 1070 m site, the emergence numbers in 2007 were recorded

from both the dissected and non-dissected pots. Therefore, a GLM test was used with 'treatments' and 'dissections' as non-dependent variables affecting the emergence of the insects.

2. GLM was used for the 450 m and 1070 m sites, 2006 collection, to test whether emergence was affected by the five different treatments. No corrections were required here as these pots of plant material were not dissected.

These GLM tests described above were done for each of the three insect species separately and were run using a binomial error distribution with Chi-square significance tests to test for adults, which emerged as adults in year 1 rather than year 2. Here I assumed there were only two possible outcomes: to emerge after one year or to emerge after two years. Bonferroni correction was used and values were considered significant if the probability was <0.017.

In Table 6.3, I present z and P values from the GLM analyses, which shows the estimated mean change of the response variable for each treatment compared to the Control group (since the Control group comes alphabetically first). This 'estimate' parameter is tested for a significant departure from zero (i.e. a difference from the Control group) by means of a z-test. If a certain group is different from the Control group, it can either have significantly higher or significantly lower emergence during year 1. Bonferroni correction was used and values were considered significant if the probability was <0.017.

6.3 Results

6.3.1 Insect Emergence

All three species had prolonged diapause with some emergence two years after collection (Figure 6.1, Table 6.1) where *E. chionochloae* and its parasitoids collected in 2004/05 emerged in different numbers in 2005/06 and in 2006/07 in each of the different sites. At the 1300 m site emergence by all three insects was low and therefore I did not analyse the data. The replicates used in this analysis were plants (pots), not insects. That suggests that *E. chionochloae* and its

parasitoids all have prolonged diapause. This prolonged diapause appears to vary among sites and years.

At the 450 m site, 2005 collection, less than 30% of the total *E. chionochloae* adults that emerged in three years came out in year 1. This year was a high flowering year with a very large number of inflorescences available for the insects' offspring to consume. During year 1 of this collection, more *Z. chionochloae* emerged in this site than *E. chionochloae* and predation levels by this parasitoid only were very high (~43%). Insects from the 2006 collection had a very high emergence rate (80%) during their first year in 2006/07 summer season (Table 6.1), although this was a low flowering year (Kelly et al., 2008). In both collections, overall predation levels by both parasitoids combined, dropped from 59% or 48% in year 1 to 29% or 28% in year 2 (2005 or 2006 collections respectively). Predation levels decreased even more during year 3 (10%) of the 2005 collection, when very few insects from all three species emerged in this low flowering year (Table 6.1).

At the 1070 m site, the insects responded quite differently to the specific conditions in that site, however the two collections were quite similar in terms of parasitism levels. *E. chionochloae* emergence from the 2005 collection was higher in year 1 than in year 2 or year 3 (66% of all adults seen in three years came out in year 1), which coincides with when there were more florets available in the field. Nevertheless, the 2006 collection had a higher emergence rate in year 2 rather than in year 1 (22% emergence in *E. chionochloae*). However, although both 2006/07 and 2007/08 were low flowering years, the 2007/08 flowering season had slightly higher flowering intensity than the 2006/07 one (Kelly et al., 2008). In both collections *Gastrancistrus* and *Z. chionochloae* emerged in higher numbers when more emergence of their host occurred and therefore more suitable hosts to parasitize and availability of food for their offspring.

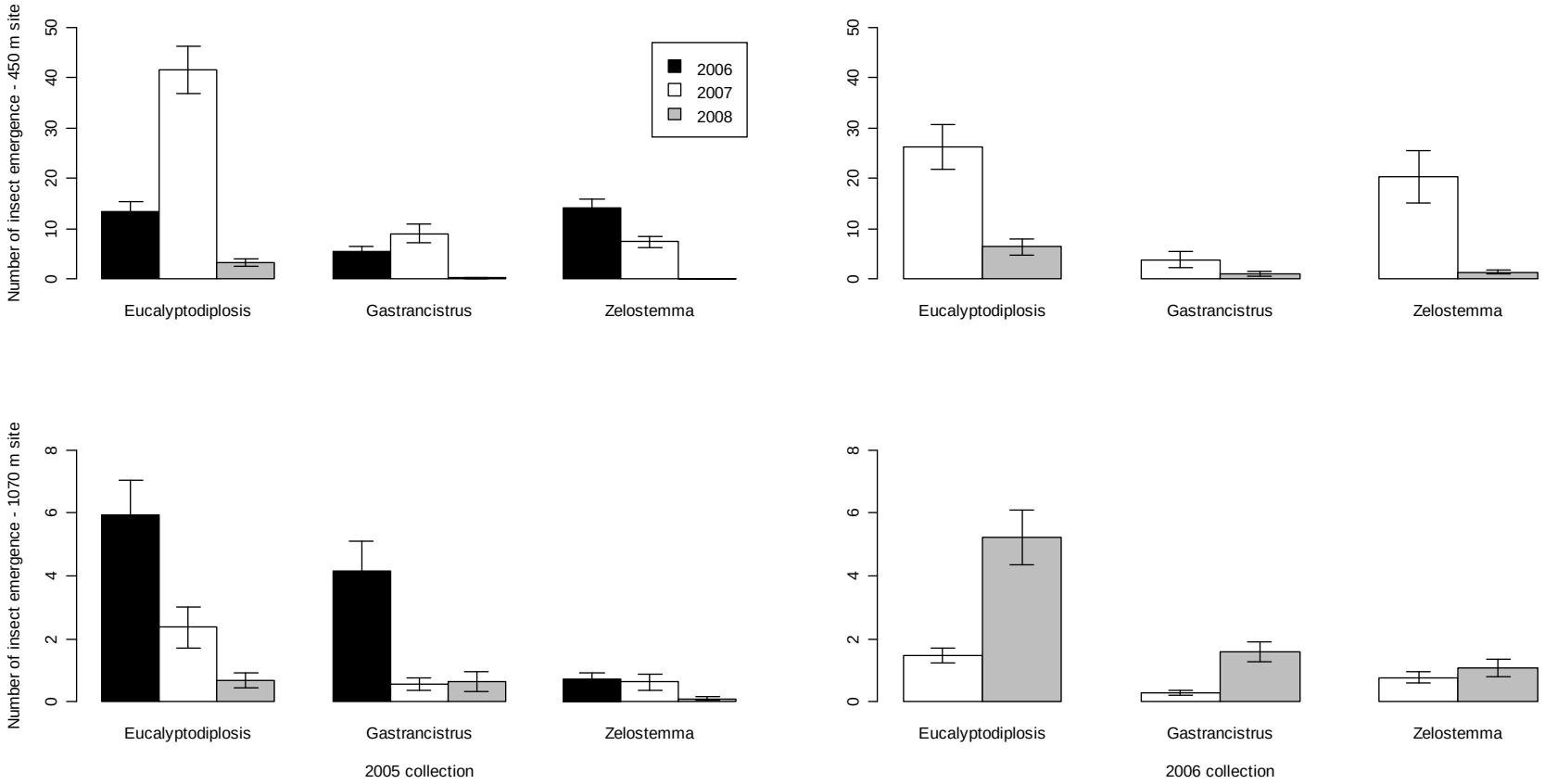

Figure 6.1 Mean number of emergence of *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* species per plant (pot) (±SE) for two altitudes, the 450 m and 1070 m sites, in three (2005 collection) and two (2006 collection) summer seasons. Abriviations: 2006, 2005/06; 2007, 2006/07 and 2008, 2007/08.

Table 6.1 Total number of emergence by the three invertebrates of the two collections in three summer seasons and three elevations. Percentages of parasitism and emergence during year 1 are also calculated. Abriviations: *Ec*, *E. chionochloae*; *G*, *Gastrancistrus*; *Zc*, *Z. chionochloae*.

			Total number of insects					% emerging year1		
Site/plant spp.	**Collected**	**Emerged**	***Ec***	***G***	***Zc***	**Total**	**% parasitism**	***Ec***	***G***	***Zc***
450 m	2005	2005/06	905	362	948	2215	59.14	28.66	42.38	71.17
(*C. rubra*)		2006/07	2060	477	377	2914	29.31			
		2007/08	192	15	7	214	10.28			
	2006	2006/07	1578	233	1222	3033	47.97	80.30	77.92	94.21
		2007/08	387	66	75	528	26.70			
1070 m	2005	2005/06	268	187	33	488	45.08	66.17	77.91	49.25
(*C. pallens*)		2006/07	107	25	29	161	33.54			
		2007/08	30	28	5	63	52.38			
	2006	2006/07	88	18	47	153	42.48	21.89	15.78	41.96
		2007/08	314	96	65	475	33.89			
1300 m	2005	2005/06	5	1	3	9	44.44	55.56	10.00	60.00
(*C. macra*)		2006/07	4	9	2	15	73.33			
	2006	2006/07	2	1	1	4	50.00			

6.3.2 Treatments and Diapause rate

6.3.2.1 Eucalypodplosis chionochloae

Treatments significantly affected the emergence of *E. chionochloae* in both collections and sites (Table 6.2). Insects from the 450 m site of the 2005 collection had more insects coming out in the first year when treated with GA_3 and pruning, but fewer insects came out in the first year when temperature was elevated. At the same site, the 2006 collection had significantly more *E. chionochloae* emergence when treated with GA_3 and a combination of pruning and GA_3. At the 1070 m site both GA_3 and a combination of warming and GA_3 significantly increased the number of insects, which emerged in the first year. The 2006 collection at the same site had more insects coming out from the warmed plants (Table 6.3, Figures 6.2-6.5).

6.3.2.2 Gastrancistrus sp.

Treatments significantly affected emergence of *Gastrancistrus* in both collections at the 450 m site but not at the 1070 m site (Table 6.2). Fewer *Gastrancistrus* came out in year 1, 2005

collection at the 450 m site when host plants were treated with a combination of elevated temperature and GA_3. The 2006 collection had significantly fewer insects coming out in the first year when host plants were treated with GA_3 and a combination of GA_3 and pruning. GA_3 at the 1070 m site had an affect on *Gastrancistrus* emergence being higher at the first year of emergence of the 2005 collection. No significant difference was detected in emergence from any of the other treated plants of the 2006 collection (Table 6.3, Figures 6.2-6.5).

6.3.2.3. Zelostemma chionochloae

Z. chionochloae emergence was affected by treatments only at the 450 m site in both collections; treatment effects were not significant at the 1070 m site (Table 6.2). All the different treatments excluding the combination of warming and GA_3, from the 2005 collection had a positive effect on *Z. chionochloae* emergence at the 450 m site. However, the 2006 collection had an increase in *Z. chionochloae* emergence in the first year only in insects originated in pruned plants. Significantly fewer insects came out from plants treated with a combination of warming and GA_3. At the 1070 m site there was no effect of treatments on insect emergence (Table 6.3, Figures 6.2-6.5).

Table 6.2 GLMs with Chi-squre test and binomial distribution for first year emergence/not of *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* species over two years for both collections using 'treatments' as the independent variable at the 450 m and 'treatments' and 'dissections' as two independent variable at the 1070 m sites. Bonferroni correction was used, therefore p-values are considered significant if they are <0.017. Significant values are in bold

Spp.	Site	Collection		Df	Deviance	AIC	LRT	Pr(Chi)
E. chionochloae	450 m	2005	NULL		1286.0	1526.4		
			treatments	5	1470.9	1701.3	184.9	**< 0.001**
		2006	NULL		295.71	440.38		
			treatments	5	348.27	482.95	52.56	**<0.001**
	1070 m	2005	NULL		96.873	169.155		
			dissections	1	99.389	169.672	2.517	0.113
			treatments	5	113.904	176.187	17.032	**0.004**
		2006	NULL		161.244	233.584		
			treatments	5	191.410	253.750	30.166	**<0.001**
Gastrancistrus	450 m	2005	NULL		442.44	559.20		
			treatments	5	458.30	565.06	15.86	**0.007**
		2006	NULL		42.049	84.423		
			treatments	5	102.778	135.152	60.730	**<0.001**
	1070 m	2005	NULL		41.496	76.070		
			dissections	1	44.817	77.391	3.321	0.068
			treatments	5	53.281	77.854	11.785	0.038
		2006	NULL		38.707	68.685		
			treatments	5	45.251	65.229	6.544	0.257
Z. chionochloae	450 m	2005	NULL		687.27	836.94		
			treatments	5	749.00	888.67	61.73	**<0.001**
		2006	NULL		164.64	231.88		
			treatments	5	227.83	285.07	63.19	**<0.001**
	1070 m	2005	NULL		31.929	58.426		
			dissections	1	32.976	57.473	1.047	0.3062
			treatments	5	36.962	53.458	5.032	0.412
		2006	NULL		48.862	89.090		
			treatments	5	55.346	85.574	6.484	0.262

Table 6.3 Summary table for the glm analyses presented in Table 6.2. Effects of treatments on diapause in *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* over 2 years at Mt. Hutt. The 2005 and 2006 collections are based on % of adults emerging over two years, which emerged in year 1. Each of the treatments is compared to the Control group. Bonferroni correction was used, therefore p-values are considered significant if they are <0.017.Abbreviations: GA_3, gibberellin A_3; P, root pruning; W, warming; W+GA_3, warming + GA_3; P+GA_3, root pruning + GA_3. Significant values are in bold.

			E. chionochloae		***Gastrancistrus***		***Z. chionochloae***	
Elevation	**Collection**	**Treatment**	**z value**	**Pr(>|z|)**	**z value**	**Pr (>|z|)**	**z value**	**Pr (>|z|)**
450 m	2005	GA_3	4.627	**<0.001**	-0.782	0.434	4.824	**<0.001**
		P	9.502	**< 0.001**	0.618	0.536	6.930	**<0.001**
		W	-2.436	**0.015**	-2.344	0.019	4.174	**<0.001**
		W+GA_3	0.043	0.965	-2.980	**0.002**	2.007	0.045
		P+GA_3	0.748	0.454	-1.368	0.171	5.230	**<0.001**
	2006	GA_3	-4.603	**<0.001**	-2.582	**0.009**	-1.101	0.271
		P	0.448	0.654	0.884	0.377	2.636	**0.008**
		W	0.596	0.551	0.004	0.997	-2.244	0.024
		W+GA_3	-0.113	0.910	0.008	0.993	-4.683	**<0.001**
		P+GA_3	-4.492	**<0.001**	-4.210	**<0.001**	0.867	0.386
1070 m	2005	GA_3	2.773	**0.005**	2.635	**0.008**	0.243	0.808
		P	0.471	0.637	0.776	0.437	0.627	0.531
		W	0.622	0.533	0.654	0.513	-0.445	0.656
		W+GA_3	2.682	**0.007**	0.006	0.995	0.007	0.995
		P+GA_3	1.656	0.097	1.854	0.063	0.844	0.399
	2006	GA_3	<0.001	1.000	1.379	0.168	2.154	0.031
		P	0.097	0.922	0.543	0.587	-0.336	0.737
		W	3.103	**0.001**	1.226	0.220	1.308	0.191
		W+GA_3	2.140	0.032	0.008	0.994	0.617	0.537
		P+GA_3	-1.706	0.088	0.867	0.386	0.999	0.318

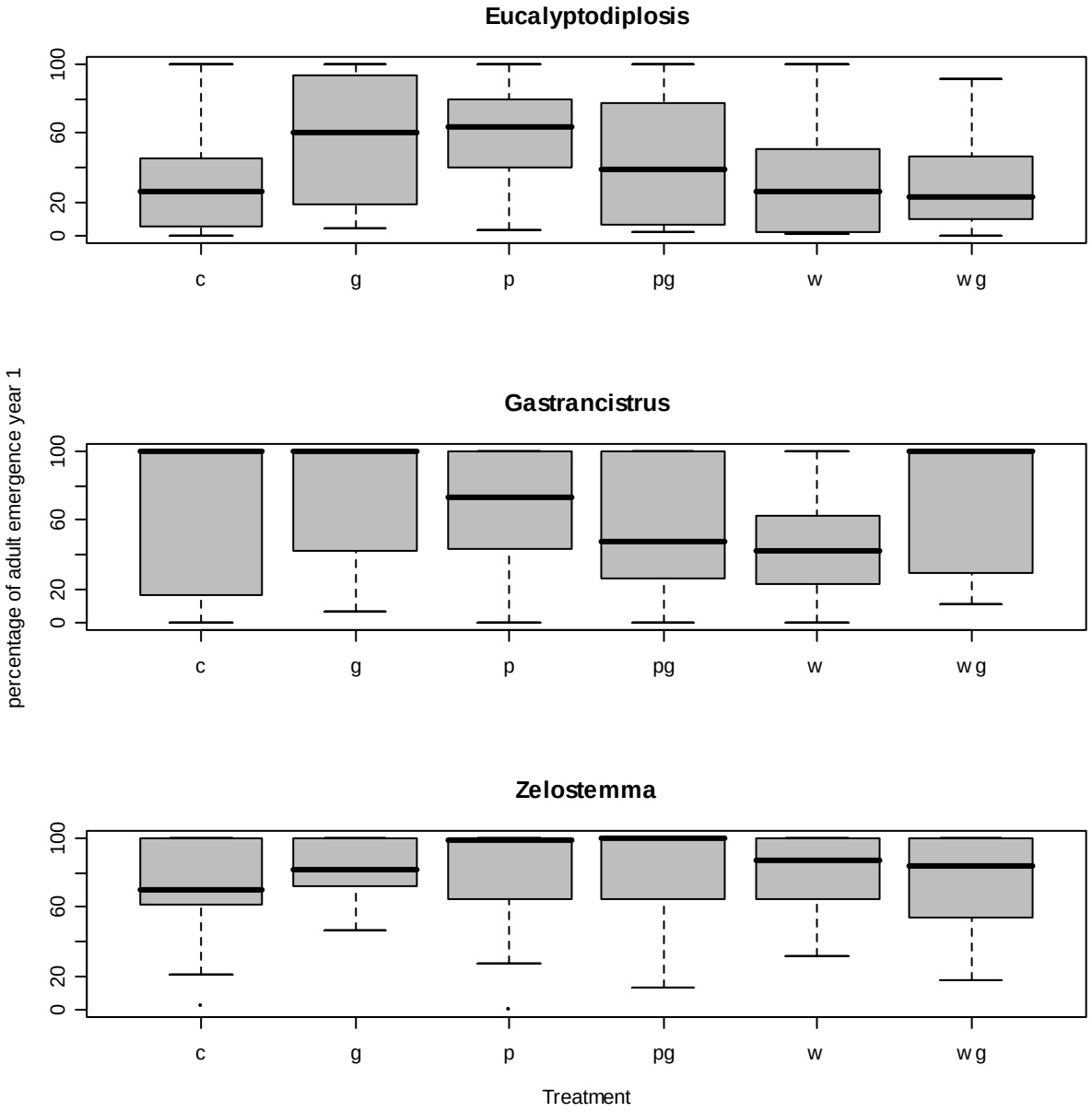

Figure 6.2 2005 collection. Percentage of adult *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* which emerged in year 1 rather than year 2, at the 450 m site. Numbers corrected for dissections. Abbreviations: c, control; g, gibberellin A3; p, root pruning; w, warming; wg, warming + GA_3; pg, root pruning + GA_3. Horizontal line = median; Bottom and top of the box = 25 and 75 percentile respectively; Whisker = 1.5 times the interquartile of the data; Outliers are drawn individually as points.

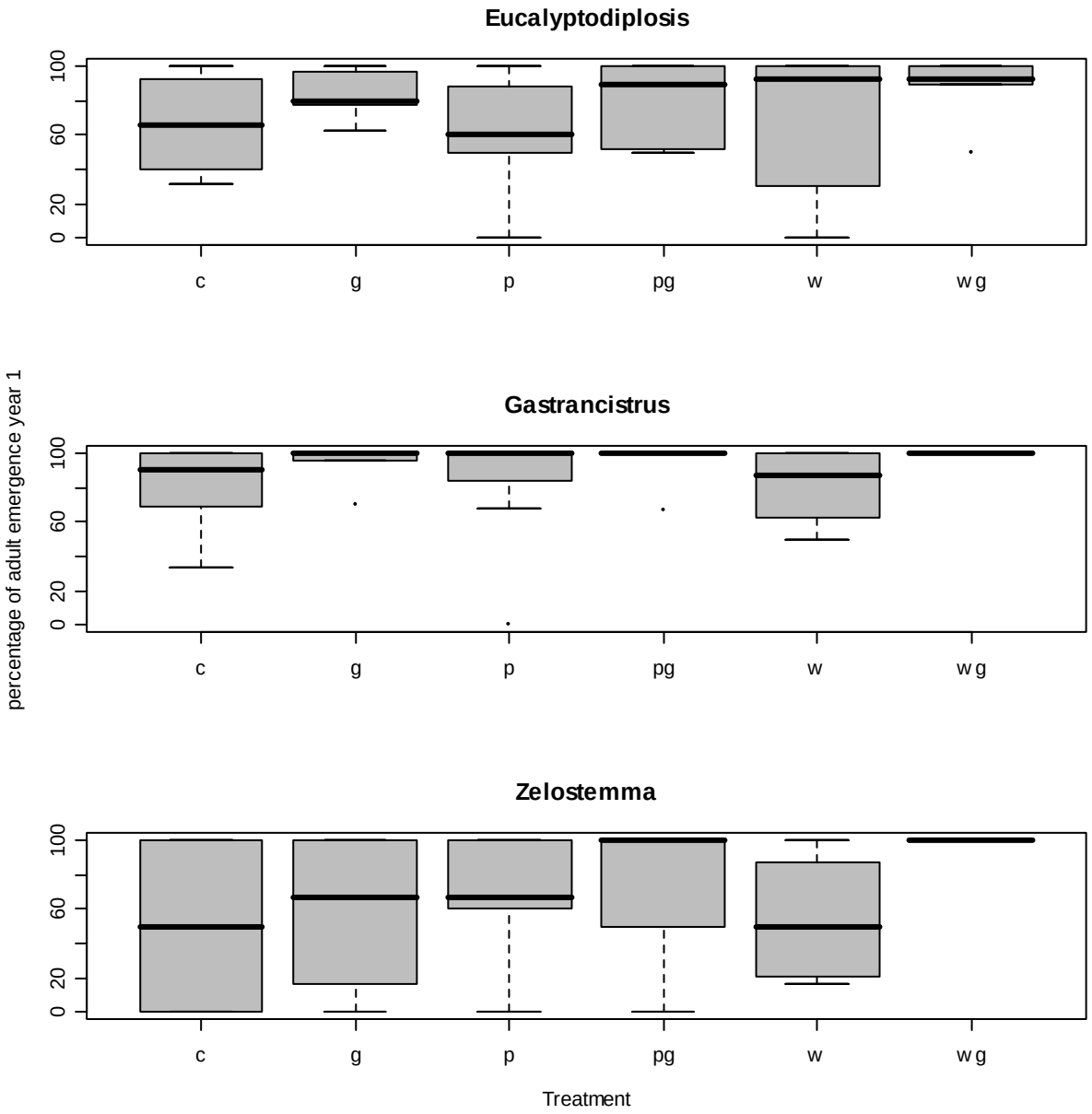

Figure 6.3 2005 collection. Percentage of adult *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* which emerged in year 1 rather than year 2, at the 1070 m site. Numbers corrected for dissections. Abbreviations: c, control; g, gibberellin A3; p, root pruning; w, warming; wg, warming + GA_3; pg, root pruning + GA_3. Horizontal line = median; Bottom and top of the box = 25 and 75 percentile respectively; Whisker = 1.5 times the interquartile of the data; Outliers are drawn individually as points.

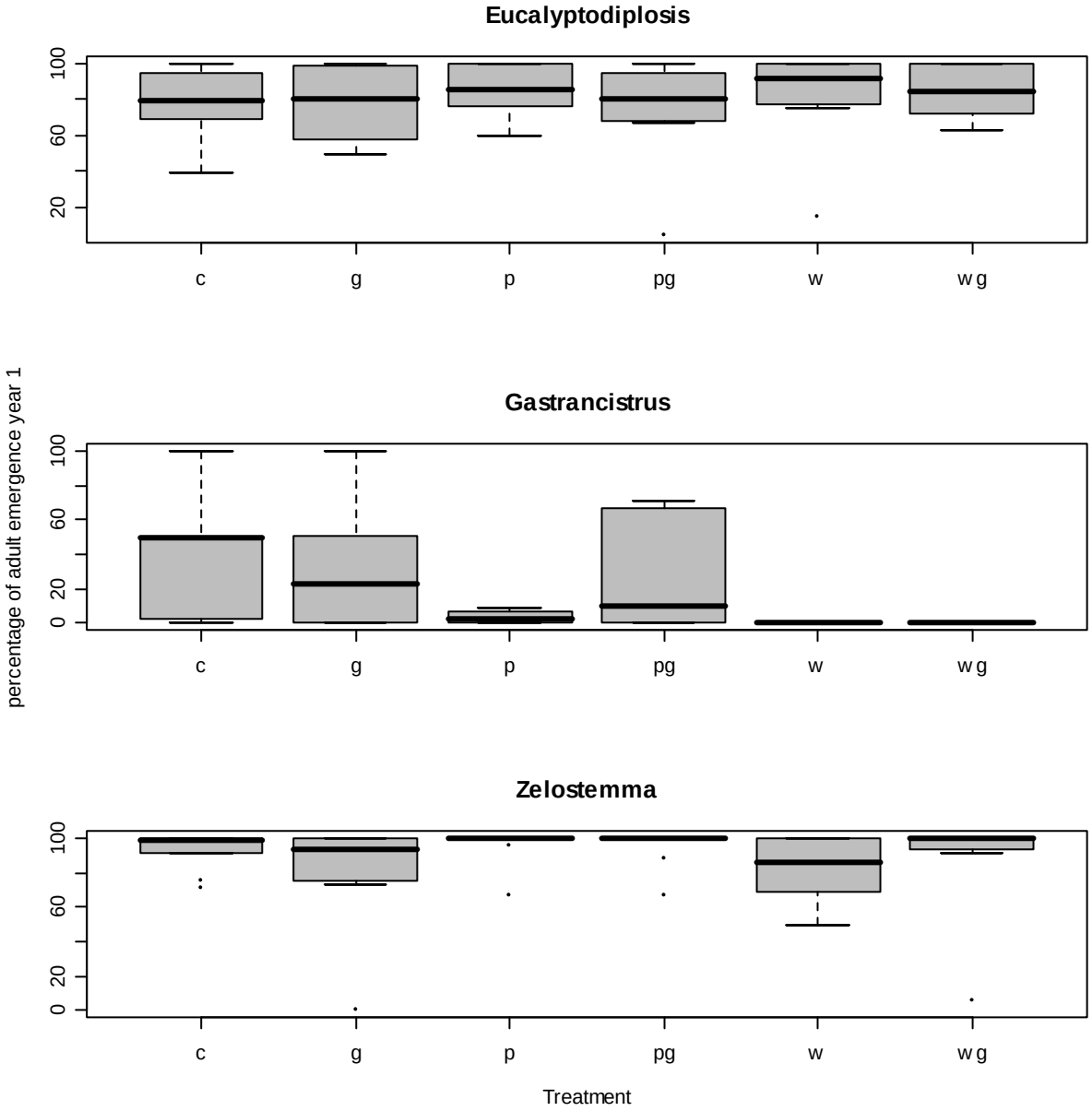

Figure 6.4 2006 collection. Percentage of adult *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* which emerged in year 1 rather than year 2, at the 450 m site. Abbreviations: c, control; g, gibberellin A3; p, root pruning; w, warming; wg, warming + GA_3; pg, root pruning + GA_3. Horizontal line = median; Bottom and top of the box = 25 and 75 percentile respectively; Whisker = 1.5 times the interquartile of the data; Outliers are drawn individually as points.

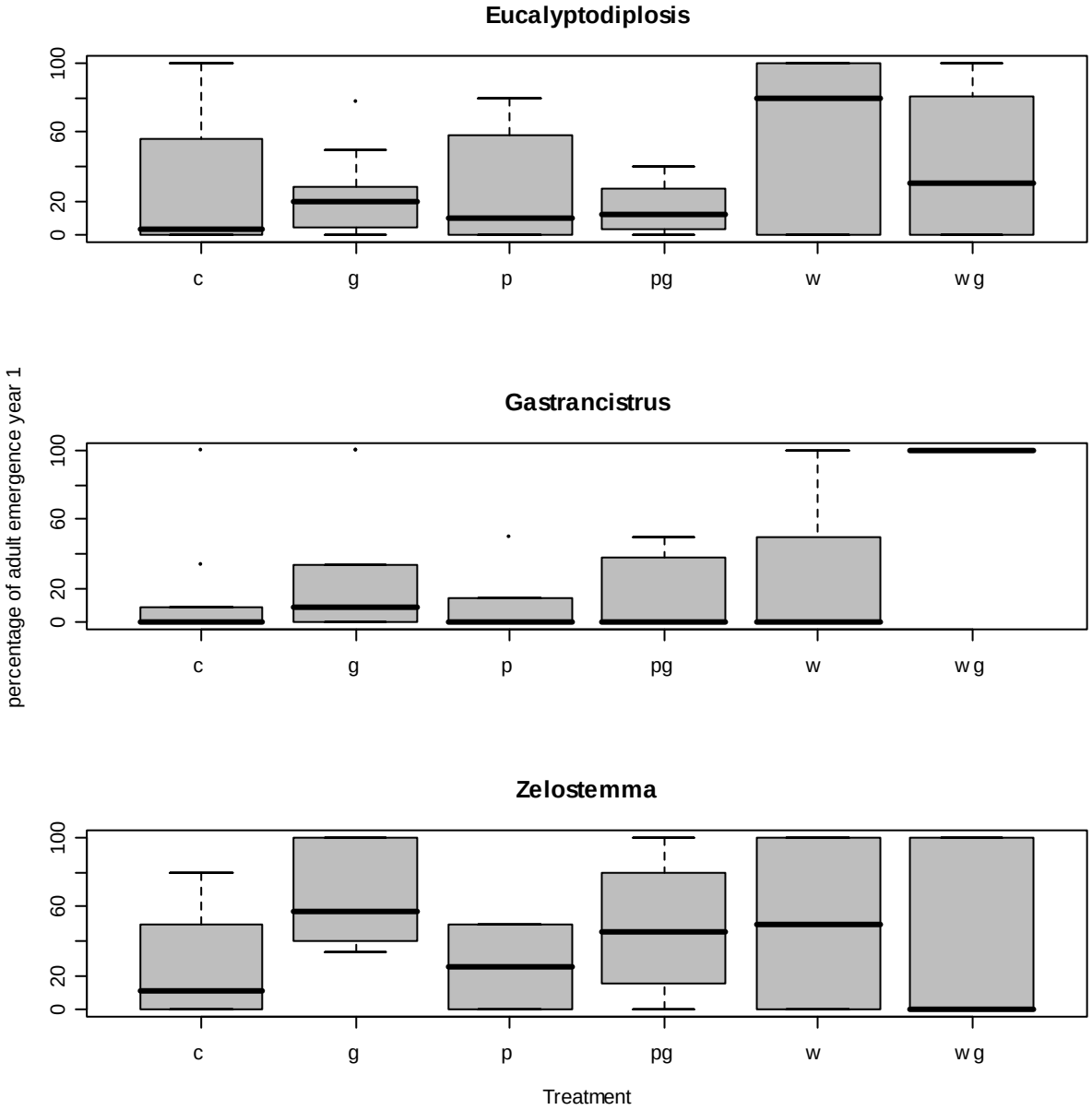

Figure 6.5 2006 collection. Percentage of adult *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* which emerged in year 1 rather than year 2, at the 1070 m site. Abbreviations: c, control; g, gibberellin A3; p, root pruning; w, warming; wg, warming + GA_3; pg, root pruning + GA_3. Horizontal line = median; Bottom and top of the box = 25 and 75 percentile respectively; Whisker = 1.5 times the interquartile of the data; Outliers are drawn individually as points.

6.3.3 *Chionochloa* flowering triggers compared with emergence/diapause

In Table 6.4, I summarize treatment effects on plant flowering intensity in the following year in two sites and two years of study. The cues *Chionochloa* used were mostly detected by one insect or more. There were some cues that the insects used to alter diapause which did not cause a flowering response in any of the elevations or seasons (i.e., warming at the 1070 m site, 2006 collection increased *E. chionochloae* emergence but its host plant did not increase

its flowering intensity in that site and collection). The parasitoids synchronized with their host but not with each other while *Gastrancistrus* seems more synchronized with its host than *Z. chionochloae*.

Table 6.4 Summary table for treatment affecting following year flower production of *Chionochloa* spp. and diapause by their insect predator and parasitoids (increase/decrease emergence in year 1). Abbreviations: +, significant increase in flowering/year 1 emergence; –, decrease in flowering/emergence; (+) or (–), marginally significant ($0.10 > P > 0.05$) increase/decrease flowering/emergence; GA_3, gibberellins A_3; P, root pruning; W, warming; WG, warming + gibberellins A_3; PG, root pruning + gibberellins A_3.

Year Treatment	**Year Emergence**	**Treatment**	***Chionochloa***		***E. chionochloae***		***Gastrancistrus* sp.**		***Z. chionochloae***	
			450 m	1070 m	450 m	1070 m	450 m	1070 m	450 m	1070 m
2005	2005/06	GA_3	+	+	+	+		+	+	
		P		+	+				+	
		W			–		–		+	
		WGA_3	+			+	–		+	
		PGA_3	+	+		(+)		(+)	+	
2006	2006/07	GA_3	+	+	–		–			+
		P		+					+	
		W				+			–	
		WGA_3	+						–	
		PGA_3	+	+	–		–			

Colour codes: same colour (across the different species) for each of the treatments was given if *E. chionochloae* followed the same cue as *Chionochloa* OR if the parasitoids (*Gastrancistrus* or *Z. chionochloae*) followed the same cues as *E. chionochloae* at the 450 m site (in purple) or at the 1070 m site (in yellow). Light blue colour was given to wasps which detected correctly the host plant behaviour but their host did not respond to the same treatment. Signs with no colour did not have a correlation between predator-host or parasitoid-host behaviour.

6.4 Discussion

6.4.1 Insect emergence over sites and years

Many seed and cone insects enter prolonged predictive diapause in order to escape the low years and maximise seed consumption in high flowering years (Annila, 1981; Hedlin et al., 1982; Hanski, 1989; Roques, 1989; Brockerhoff & Ho, 1997; Brockerhoff & Kenis, 1997; Maeto & Ozaki, 2003). Predictive diapause was also found in hymenopteran parasitoids (Claret & Carton, 1980; Annila, 1981; Brodeur & McNeil, 1989; Polgar & Hardie, 2000; Garcia et al., 2002). Prolonged diapause in host and parasitoid may complicate the dynamics of the system where each species struggles to synchronize with its host but desynchronize with its predator (Hanski, 1989). Different species of parasitoids from the same trophic level, which share the same host, may also be selected to desynchronize with each other in order to escape competition. This is probably the first time that the dynamics of three trophic level

mast seeding plants and their diapausing insects have been studied simultaneously with the factors which control their population fluctuations by environmental and plant cues.

In general at Mount Hutt, the total number of insects emerging was smaller with increased elevation, which was at least partly because plants at lower elevations were larger and had more flowering material per plant. Parasitism rates in the two lower altitudes were relatively high, especially in the first year of adult emergence. The 1300 m site produced very few insects, which are not sufficient for analysis and discussion, and therefore I am not going to refer to this site further. There was a difference in emergence between my two study sites and the two different collections in terms of insect emergence.

Populations from different geographical areas show different responses to the same environmental stimuli to adapt themselves to the specific biotic and abiotic conditions they experience in their habitat (Bradshaw & Holzapfel, 1983). For example, Krraijeveld and van der Wel (1994) found a great geographical variation in the probability of survival of the *Drosophila* parasitoid *Asobara tabida,* where insects from south Europe survived in higher rates than their conspecifics from north, west or central Europe. Altitude in particular, was found to be an important factor in these adaptations and specifically the life histories of insects change according to the local conditions they face in these different elevations (Leather et al., 1993). For example, Bradshaw (1976), showed that the mosquito *Wyeomyia smithii* synchronizes its emergence from diapause according to local photoperiods which differ across altitudes and latitudes.

At Mt. Hutt, the differences between the two study sites can presumably be attributed to abiotic factors (e.g., the differences in altitudes and hence temperature, the different environmental conditions) and biotic factors (e.g., plant species, plant densities, abundance of interspecific competitors, flowering intensity and hence availability of food supply and sufficient feeding by the seed predator and parasitoids) in each of these sites and years.

6.4.2 Plant cues and diapause

According to Turnbull et al., (in prep), both species of *Chionochloa* responded to GA_3 and combinations of GA_3 with any other treatment. Other grass species, such as *Lolium temulentum* and *Bryophyllum* spp. are known to induce flowering as a response to GA_3 (Pharis & King, 1985; Evans, 1999; King et al., 2006). Populations of insects that experience biotic and abiotic factors which may have chaotic, unpredictable dynamics (e.g. unavailability of

host or unsuitable temperatures), may be selected to delay emergence (Kraaijeveld & Vanalphen, 1995) to coincide with their hosts. Smith and Balda (1979) suggested that because monophagus parasitic wasps are exposed to the same problems as their hosts in finding their food, (which may be scarce in some years), the unique ways these different insects obtain their resources can be very similar. Therefore parasitoids may have an independent induction of diapause which is not related to their host's physiological condition. Such independent diapause occurs in the parasitoid of the Argentine stem weevil, *Microctonus hyperodae* Loan (Hymenoptera: Braconidae, Euphorinae), and was found to be related to photoperiod independently in both host and parasitoid (Goldson et al., 1993). Temperature is considered to be a key factor inducing diapause during development in the immature stages. For example, Garcia et al., (2002) suggested that the parasitoid species, *Trichogramma cordubensis* arrests the development of their immature stages as a function of low temperature; Brodeur and McNeil (1994) studied diapause in the prepupal stages of the parasitoid *Aphidius nigripes* and found that the incidence of diapause increases with decreasing temperatures. Other studies found similar trends of diapause as a function of temperature (Roques, 1989; Tsukada, 1999; Polgar & Hardie, 2000).

Both *Gastrancistrus* sp. and *Z. chionochloae* adapted to use broadly similar cues to their host insect (prey) and to their host plant to adjust their diapause. In general, *Z. chionochloae* used more cues at the 450 m site than at the 1070 m site which adds to the claim that it is more successful at lower elevations (Chapters 3 and 5). In addition it responded to many more cues than the other two insects. *Gastrancistrus* responded to cues from both the 450m and the 1070 m sites and in general was in synchrony with *E. chionochloae* or with *Chionochloa* plants. Usually, *Gastrancistrus* better synchronized with *E. chionochloae* than *Z. chionochloae* (Table 6.4), where higher numbers of *E. chionochloae* correlated with higher numbers of *Gastrancistrus* and vice versa. *Z. chionochloae* however had weak positive correlation with *E. chionochloae*. *Gastrancistrus* may use its host physiology as cues and may also have better prediction abilities than *Z. chionochloae*. However, although both parasitoid species used at least some of the cues *E. chionochloae* used, not all their emergence responded in parallel with that of their host. For example, *E. chionochloae* from the 2005 collection used warming in the 450 m site as a cue to **reduce** emergence while *Z. chionochloae* used the same cue at the same site and year to **increase** its emergence. Additionally, many studies showed that *Chionochloa* spp. use temperature of the previous-year summer season as a cue for flowering (Webb & Kelly, 1993; Kelly, 1994; Kelly & Sullivan, 1997; Kelly et al., 2000; Rees et al., 2002; Schauber et al., 2002; Kelly et al., 2008). However, despite the abundant literature

showing a previous-year effect of warm summers on flowering, no such effect was detectable following experimental warming by Kelly et al., (2008) although they again showed a strong previous-year effect observationally in a 22 year dataset. *E. chionochloae* had a significantly higher emergence rate as a response to plant warming only once, the 2006 collection at the 1070 m site. This however was not followed by its parasitoids, which did not respond to my warming treatments in this site, year and collection. Additionally, *E. chionochloae* had significantly **lower** levels of emergence when subjected to warmer temperatures of the 2005 collection, at the 450 m site, although Kelly et al., (2008) and Turnbull et al., (in prep) did not find a previous year effect from the same warming treatment. Nonetheless *Gastrancistrus* synchronized with its host and had lower emergence under these conditions, while *Z. chionochloae* increased its emergence. Perhaps the amount of warming from the warming tubes was insufficient to promote next year flowering as well as increase the level of insect emergence in *E. chionochloae* and *Gastrancistrus* sp. The plastic tubes increased humidity of the air surrounding the plant (change in environmental conditions) and could also serve as a physical barrier to the insects and females may have chosen to oviposit on another plant. However, the significantly higher emergence of *Z. chionochloae* (2005 collection, 450 m site) and *E. chionochloae* (2006 collection, 1070 m site) under the warming treatments in comparison to their specific control groups suggest that the females of *E. chionochloae* and *Z. chionochloae* were capable of finding the florets in spite of the difficulty in accessing the plants.

Root pruning increased *C. pallens* flowering at the 1070 m site during the first flowering year of the 2005 collection. At that site the insects did not respond to the root pruning treatment, however *E. chionochloae* and *Z. chionochloae* increased their diapause at the 450 m site of this collection (although *C. rubra* did not increase its flowering intensity as a response to pruning). Root pruning may have caused changes of the biotic and abiotic conditions of the plants (e.g., lower available resources other than water, reduced root-herbivory and reduced root competition between different individuals of the same species). These changes in plant conditions may have affected the plants and the insects which feed on their seeds in different ways which may explain the inconsistency of these results.

The combination of root pruning and GA_3 was better detected by the plants and insects in the 2005 collection, 450 m site (Table 6.4). GA_3 alone or combined (with root pruning or warming) was usually detected by both species of plants. The insects mostly were able to detect these cues, but not always in the same direction (i.e., *E. chionochloae* and

Gastrancistrus sp. from the 2006 collection **reduced** their first year emergence as a response to **increased** flowering intensity at the 450 m site).

These inconsistencies of emergence and flowering in the experimental plants and insects may have to do with one or both of the following: (i) the insects did not respond to the treatments applied to plants and the emergence of the insects was affected by other factors; (ii) the insects were subjected to the treatment applied while they were attached to their host plant (current year) rather than when they were away from the plant, diapausing in the florets because I hypothesized that they receive their signals while they feed. However, warming and GA_3 treatments in *Chionochloa* plants were shown to have current-season effects on flowering (Kelly et al., 2008; Turnbull et al., in prep., see appendix). Therefore, the timing of the received cues was not in synchrony between the plants and insect.

6.4.3 Predictive or risk-spreading (bet-hedging) diapause?

E. chionochloae may use risk-spreading diapause, predictive diapause or a combination of the two to survive the harsh environment they have to cope with. In the last 22 years, only four years had heavy flowering by *Chionochloa* spp. (Figure 1 in Kelly et al., 2008). Most of the other years had moderate to low flowering effort. Using only a risk-spreading strategy for emergence by the insects may not be advantageous because of the lack of food supply in most years. If *E. chionochloae* used only risk-spreading diapause it could face a higher risk of extinction.

E. chionochloae may use predictive diapause and have the ability to detect plant cues while they feed on the seeds, however after florets are no longer attached to the plants, these cues can be no longer detected and the insects should use other cues to control their diapause. Climate cues, such as temperature, photoperiod and humidity can be detected by the insects without being physically attached to the host plant. A combination of plant and climate cues would provide accuracy in prediction both while on the plant and after the florets (with enclosed larvae) have fallen from the mother plant. All three insects from plants treated with a combination of warming and GA_3 in the 2005 collection increased or decreased their emergence significantly in comparison to the Control group, however there was no real pattern for these differences in emergence/diapause. *E. chionochloae* had higher emergence at the 1070 m site, *Gastrancistrus* had lower emergence at the 450 m site and *Z. chionochloae* increased its emergence at the 450 m site.

Another possibility for the diapause strategy of these three insects is the combination of risk-spreading and predictive diapause. The insects may use predictive diapause for some years and risk-spreading in others. This combined strategy may be used by insects which have already been in diapause for several years but did not detect a proper cue for emergence.

Other factors may have influence emergence by the three invertebrates: some insects may emerge before others because they may have not fed sufficiently during their feeding stage and therefore their body fats and energy reserves are quite low. Such insects will have to take the chance and emerge whether there will be sufficient food for their offspring or not. Another explanation for a combined strategy can be an insurance group. For example, Brodeur and McNeil (1989) found that the potato aphid parasitoid, *Aphidius nigripes* (Hymenoptera: Aphidiidae), has a predictive diapause and is using temperature and photoperiod to adjust its diapause. However, there was a variation in emergence between individuals in the population and emergence sometimes occurred without the signals which normally increased emergence. They suggested that this behaviour is important in the population level, against catastrophes which are unseasonable climate conditions. This suggests that together with a predictive diapause, this population was experiencing risk-spreading diapause and although there may be costs to the individuals, when emergence is unsynchronized with food availablity, some florets may still be found, and therefore in the population level the costs are reduced by an insurance group.

Another possible reason can explain the variation in insect emergence across the different treatments, which did not follow a clear pattern of predictive diapause. There may be other reasons for the insects to emerge under certain conditions which I did not test for. Such possible reasons include the maternal effect on diapause. Mousseau and Cox (1998) suggested that diapause of the progeny of females can be influenced by biotic (e.g. quality of food or quality of mate) and abiotic factors (e.g. temperature, pH, or photoperiod). According to Mousseau and Dingle (1991) and Mousseau and Cox (1998) the environmental cues that the female is subjected to in the present, serve as good indicators for the future conditions her offspring will experience. According to these cues the female will choose whether her offspring will enter diapause or have direct development. However, because *E. chionochloae* are univoltine and overwinter as larvae at least one winter, mothers will have to use temperature cues of the current season rather than future seasons and that may not be sufficient for good predictions. Although relatively few studies have tested the influence of temperatures that the females experience and the length of diapause of their progeny,

temperature has been found to serve as a primary cue in maternal induction of diapause (Mousseau & Dingle, 1991). For example, females of the mosquito *Aedes togoi* produced a higher proportion of diapausing eggs when they were subjected to low temperatures (Vinogradova, 1975); Females of the parasitoid wasp *Trichogramma evanescens* produced lower proportions of non-diapausing larvae in higher temperatures. In my experiments I did not test for maternal effects on insect diapause and all treatments were applied after oviposition occurred (i.e. were applied to larvae).

To conclude, this study shows that all three species use prolonged diapause. Insects responded to the treatments by reducing or increasing their emergence rates and also differed in their emergence rates across sites and plant species. A predictive diapause would be selectively advantageous for *E. chionochloae* and its parasitoids, which are subjected to the same chaotic and unpredictable environment in terms of food supply. However, in this study although insects responded to the treatments applied to their host plants, it was hard to draw a specific conclusion to which cue exactly they responded and I could not confirm the existence of predicted diapause. The emergence not always perfectly synchronized with the plant behaviour and some emergence or diapause patterns are not clear. Also not all insects respond to the same cues at the same elevations or time as their hosts, but all insects share some cues with their host insect or with their host plant. I suggest that there may be a combination of predictive and risk spreading diapause and that on top of that there are other unknown biological and environmental factors which may affect the insects' decision whether to emerge or not.

7. Sex ratios and Predictive Diapause in *E. chionochloae* and its Parasitoids *Gastrancistrus* Sp. and *Z. chionochloae*

7.1 Introduction

Different factors may affect the population sex ratio of phytophagous insects and their parasitoids. Changes in biotic and abiotic conditions were found to be important in sex ratio of different insect species (Mousseau & Fox, 1998). Biotic changes can be explained by the plant quality hypothesis, which suggests that plant growth may affect the fitness of male and female insect-herbivores in different ways (Craig et al., 1992). For example, populations of the hymenoperous tenthredinid sawfly *Euura lasiolepis* were male-biased on slow growing Willows (*Salix lasiolepis*), while female-biased on plants with rapid shoot growth (Craig et al., 1992). Both biotic (e.g., fast growing shoots of host plant) and abiotic factors (e.g., season) also modified the sex ratios of *Izeniola obesula* (Diptera: Cecidomyiidae) (Dorchin & Freidberg, 2004). Sex ratios are influenced by biotic and abiotic factors in parasitic insects as well. In her review, King (1987) presented offspring sex ratios for many wasp species, which decreased the proportion of female with different abiotic and biotic factors, such as extreme temperature, photoperiod, humidity, host size, maternal age at ovipositing and more. Kfir and Luck (1979) reared females of two Hymenopteran species, *Aphytis melinus* and *A. lingnanensis* in artificial high temperatures. They found that females of both species had fewer daughters than sons. Wilkes (1959) found that exposure of pupae of the parasitoid wasp *Dahlbominus fuliginosus* to high temperatures also reduced the proportion of females since fewer female pupae survived the high temperatures. However, exposure of larvae of these insects to high temperatures reduced the proportion of males as fewer male larvae survived the high temperatures. Other studies found that the sex ratio of parasitic Hymenoptera was influenced by biotic factors. Among these are host size (Kazmer & Luck, 1995), multiple mating (Allen et al., 1994) and vitamin E concentrations (Coskun et al., 2005).

Sex ratios in populations of insects may be affected by the duration of the development rate to reach the final adult stage, i.e. whether diapause is present or not (Brockmann & Grafen, 1992; Nylin et al., 1995). Differences between sexes in their

response to diapause cues can take a variety of forms. Some insect species were found to have a heavily male-biased sex ratio in emergence after prolonged diapause. That is, sex ratio may have been female biased in the first year of emergence for the insects in a population which used simple diapause rather than prolonged diapause. For example, the mosquito *Toxorhynchitis rutilus* (McCrary & Jenner, 1965); the flesh flies *Sarcophaga* (Denlinger, 1972b, 1972a); the blow fly *Lucilia caesar* (Ring, 1971) and the *Drosophila* parasitoid *Asobara tabida* (Kraaijeveld & Vanalphen, 1995). This skew in sex ratio is usually attributed to the higher costs females have to pay for staying in prolonged diapause (i.e., loss of body weight over the winter), while males may increase their fitness by emerging earlier than females in the following summer and increasing their chances to mate (protandry, see below) (Brockmann & Grafen, 1992; Nylin et al., 1995). However other insect populations, such as *Anthocoris tomentosus* and *A. antevolens* (Heteroptera: Anthocoridae, Miridae) showed a higher incidence of diapause in females (Horton et al., 1998).

Emergence patterns of adult insects, like many other morphological and behavioral characteristics, are adapted to local conditions (Tauber et al., 1986). Selection may change developmental rate before and after diapause and influence emergence pattern (Leather et al., 1993). Darwin (1871) hypothesized that the pattern of male emergence before female had evolved by natural selection, operating on the individual level and that by this behavior males are likely to have an advantage in competition for mates. The term 'protandry' has been used in recent years to describe this pattern of emergence (Wiklund & Fagerstrom, 1977) and occurs when males emerge slightly before females in the season. This phenomenon occurs particularly in species where the females mate only once in their lifetime shortly after emergence and the males are short lived (Wiklund & Fagerstrom, 1977; Fagerstrom & Wiklund, 1982; Hastings, 1989). Protandry occurs mainly in butterflies (Wiklund & Fagerstrom, 1977; Hastings, 1989) and solitary Hymenoptera (Gwynne, 1980). Many studies showed protandry by insects, Brockmann (2004) for example, studied adult emergence patterns in diapausing and non-diapausing male and female of *Trypoxylon politum* (Hymenoptera : Sphecidae). She found that males emerged slightly before females in the diapausing population whereas in the non-diapausing population there was no difference in emergence patterns. Takeda and Chippendale (1982) found that females

of the Southwestern corn borer *Diatraea grandiosella* under laboratory conditions resume active development after diapause earlier than males. Under field condition however, there was no difference in timing of emergence between males and females.
E. chionochloae (Diptera: Cecidomayiidae) is a seed feeder specific to the alpine tussock grasses from the genus *Chionochloa* (Poaceae) which dominant the high countries of the South Island of New Zealand (Kelly et al., 2000; McKone et al., 2001; Kolesik et al., 2007). *Chionochloa* spp. experience pronounced mast seeding (a highly variable and synchronous flowering among years (Kelly, 1994; Kelly et al., 2000)) which has been shown to mainly benefit from predator satiation (Kelly & Sullivan, 1997; Sullivan & Kelly, 2000; Kelly et al., 2001). Apart from *E. chionochloae*, two other seed and flower predators are known to be feeding on *Chionochloa* (McKone et al., 2001). These are *Megacraspedus calamogonus* Meyrick (Lepidoptera: Gelechiidae) and *Diplotoxa similis* Spencer (Diptera: Chloropidae). Both *M. calamogonus* and *D. similis* are univoltine (McKone et al., 2001), whereas *E. chionochloae* is univoltine, all larvae enter obligatory diapause of one winter while other individuals can emerge after two winters and more (Chapter 2). *E. chionochloae* has two specific hymenopteran parasitoids, *Gastrancistrus* sp. (Pteromalidae) and *Z. chionochloae chionochloae* (Platygastridae); both species enter prolonged diapause (Kolesik et al., 2007; Buhl et al., 2008; Sarfati et al., in prep). Populations of *E. chionochloae* have been previously shown to have a female-biased sex ratio (Kolesik et al., 2007). Other cecidomyiid species have been observed to have a female-biased sex ratios, for example *Sitodiplosis mosellana* and *Contarinia tritici* (Kirby) (Smith et al., 2004 and references therein), *Izeniola obesula* (Dorchin & Freidberg, 2004) and other cecidomyiidae (Matuszewski, 1982). Female-skewed sex ratio in cecidomyiid species was suggested to have a selective benefit, for example if each male can mate with more than one female, a female-skewed progeny would increase the mother's fitness (Stuart & Hatchett, 1991).

The purposes of this chapter are (1) to find out whether sex ratios were skewed in the parasitoids of *E. chionochloae*; (2) to determine the effects of diapause on the sex ratio of each of the three species; (3) to find out whether sex ratios of all three species are affected by external conditions (environmental conditions, plant physiology or

combinations of the two); and (4) to find out whether the three species have protandry that is influenced by the diapause length.

My hypotheses were that females face higher fitness costs from diapause than males, because extended diapause presumably consumes metabolic energy that would reduce the eventual number of eggs a female could produce, more than it would reduce the ability of males to inseminate females. Therefore I predicted that (1) More males will undergo prolonged diapause than females and therefore the sex ratios will be less female skewed with increased diapause length; (2) Females will respond more strongly than males to cues which increase flowering after regular diapause (year 1) and thus the sex ratio will be more female-biased under treatments which increase insect emergence in year 1.

7.2 Methods

7.2.1 Study site

The study area is located on Mount Hutt in Canterbury, New Zealand, on the eastern edge of the central Southern Alps, approximately 110 km west of Christchurch. Two different sites at different elevations were studied: **450 m site:** located at the bottom of the mountain (43° 33.93' S, 171° 33.26' E), with *Chionochloa rubra* surrounded by exotic grasses. For more information, see also Buhl et al., (2008). **1070 m site**: located half way up the skifield road (43°32.04´S, 171°32.97´E) dominated by 94% *C. pallens* and 6% *C. macra* (Kelly & Sullivan, 1997; Kolesik et al., 2007).

7.2.2 Plant manipulations

In order to find out whether different biotic and abiotic conditions affect sex ratio of *E. chionochloae* and its parasitoids, ten replicates of the following treatments were applied over two *Chionochloa* species in two elevations, the 450 m site (*Chionochloa rubra*) and the 1070 m site (*C. pallens*) in two consecutive years: 2004/05 and 2005/06 summer seasons with the following treatments:

1. **Untreated plants – control group (C)**
2. **Plants treated with warmer temperatures (W).** Transparent open-topped plastic tubes were placed on top of the plants from December to April of each

of the 2004/05 and 2005/06 summer seasons. The plants were subjected to natural photoperiod and humidity. For more details see Chapter 6 and (Kelly et al., 2008)

3. **Plants subjected to water stress by root pruning (P).** Roots of treated *Chionochloa* plants were pruned on late December 2004 to early January 2005 (2004/05 summer season) and mid December 2005 (2005/06 summer season) using a shovel, which was inserted into the soil around half of the plant base at a 45° angle to the centre of the base of the plant.
4. **Plants treated with plant hormone gibberellic acid GA_3 (G).** Plants were treated with GA_3 (obtained from Professor Zhou Xie, Nanjing Agricultural University, China) at 150 ppm in an aqueous solution containing 0.1% surfactant (LI-700, Loveland Industries). Plants were sprayed to drip-off two times in the 2004/05 summer season (early and late January 2005). However, in the 2005/06 summer season plants were treated with GA_3 only once (late December 2005). In both years spraying was done using a plastic tube to prevent overspray of gibberellins onto other plants.
5. **Plants treated with both warm temperatures and GA_3 (WG).**
6. **Plants treated with both root pruning and GA_3 (PG).**

These treatments were a mixture of environmental cues (W to simulate warmer temperature during summer and P to simulate drought), plant cues (GA_3 is a plant hormone that induces flowering) and combinations of environmental and plant cues (WG and PG). Treated plants were expected to flower at a higher rate than the control group.

This experiment set in 2004/05 is named throughout the chapter the 2005 collection whereas the one done in 2005/06 is named the 2006 collection.

7.2.3 Collection

Insects were allowed to oviposit and feed in the inflorescences naturally. In late February 2005 inflorescences from treated plants were collected from the 450 m and 1070 m sites and each placed in different emergence traps on site. The emergence traps at the 450 m site were placed no more than 20 m away from the plants in a sheep-proof area and the traps from the 1070 m site were placed no more than 1 m

away from the plant which the insects were collected from. Insects were collected weekly from early October to early March in each of 2005/06, 2006/07 and 2007/08 flowering years. Collected insects were brought to the lab, identified to species, sexed and preserved in 70% ethanol.

Ideally, sex ratio should be measured in the egg or larva stage. However, sexing pre-mature stages without killing the larvae is very hard in *E. chionochloae*. Sexing in some species is done by weighing the larvae (females should be heavier than males, Alain Roques, per. comm.). However, in *E. chionochloae* the larvae are protected inside the florets and extracting them from the floret may damage or kill the insects (see below). Additionally, parasitized larvae may have different mass and sexing parasitoid larvae still in their host, without killing both the host and the parasitoid is impossible. Therefore I did not sex the larvae of *E. chionochloae* or its parasitoids. Instead I sexed the insects in their adult stage.

Overall during three years of insect collection, more than 10,000 insects from all three species and both sexes were observed at the two sites, of these, 8739 from the 450 m site and 1348 from the 1070 m site.

7.2.4 Dissections

When emergence of insects from the 2005 collection finished in autumn 2006, half the pots in each treatment at the 450 m and 1070 m sites were randomly selected and taken to the lab for dissection of inflorescences to count the number of larvae still in extended diapause. All material in each pot from the 1070 m site was dissected, whereas the material in each pot from the 450 m site was divided in half and only one half was dissected. The other half of the material from the 450 m pots was returned to the clay pot in the field. That was done because there was abundant material in the pots at the 450 m site. Pots were regularly watered in the lab to keep plant material and insects moist. *E. chionochloae* larvae found in each pot were counted and placed live in a new clay pot with mesh bag and soil. All pots were placed back in the field for another emerging season. These new pots were named with the same code as the original pot with the addition of the letter ‘B’ to indicate this plant material had been dissected.

Some of the larvae were accidentally stabbed during dissection and died, and these animals were not counted in the number of live larvae which were recorded as placed back into the field. Dissections also apparently did damage to the insects from two other causes. First, many insects suffered from the dry conditions at the lab and although were watered regularly, many got dry, shrank and died. Second, insects that survived dissections were removed from their floret and placed in the soil without the floret's protection, which may have decreased their survival. Therefore in the analysis of insect from the 450 m site we used only insects emerging from the half pots which were not dissected, multiplied by 2 to estimate for the half plant material which was missing (dissected) and by that correct for dissection. We also used the pots which were left in the field untouched (these were not corrected for dissections as we did not dissect plant material for these ones). As all plant material of the dissected pots at the 1070 m site were used, we included emergence data from these pots allowing dissected/not as a factor in the analysis and pots which were left untouched in the field.

7.2.5 Data Analysis

In order to test our first hypothesis, the percentage of female emergence was calculated relative to the total adults emerging in each pot (Table 7.1). Total numbers of insect emergence at the 450 m site were corrected for both males and females for dissections, but this did not affect the proportions of females.

A proportion χ^2 test was applied to the counts of male and female insects in order to test whether the sex ratios of *E. chionochloae* and its two parasitoid species differed significantly from 50:50 in each of the sites and collections (Table 7.1). Bonferroni correction was done, therefore values were considered significant if the probabilities were <0.017 (0.05/3 = 0.017). Analysis was done for each species separately assuming that each individual in the population is an independent sample. This assumption is needed as there is no information about whether *E. chionochloae* is monogenous and the oviposition behavior of the females is unknown (see section 7.4.2 for more discussion).

A Pearson's χ^2 test (Statistix version 8, Analytical Software, Tallahassee, Florida) was used for the counts of male and female insects found in the three or two years of emergence in each of the 2005 or 2006 collections respectively in order to find out whether the proportion of females differed between the different years of emergence, or in other words, whether extended diapause reduced female bias in the sex ratios (more males). I ran separate tests for each of the insect species from both collections in each of the 450 m and the 1070 m sites. Analysis was done with the assumption that each individual in the population is an independent sample (see section 7.4.2 for more discussion).

In order to check my second hypothesis, the effect of treatment on sex ratio was measured running Mixed Effect Models (R version 2.3.1 (R Development Core Team, 2005)) to test whether the sex ratio of each of the three insects was affected by year of emergence and the five different treatments applied to plants, plant was used as a random effect. Numbers at 450 m site, 2005 collection were corrected for dissections. I used 'female' and 'male' counts of emergence per pot with a binomial distribution and logit link function. Finally, coefficient estimates from each model were back transformed to get a mean abundance of insect per inflorescence and high and low Confidence Intervals. Analysis was done for sites, collections and insect species separately (Table 7.2).

7.3 Results

7.3.1 Sex ratios of the three insects

Both males and females of *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* were found to have diapause of two years or longer. The sex ratios of *E. chionochloae* and its parasitoids from the two collections were found to be different across sites (elevations) and between years of emergence.

Sex ratios of *E. chionochloae* in both collections and years of emergence were always female-biased, significantly so at the 450 m site and mostly significant at the 1070 m site (Table 7.1). Sex ratios of *Gastrancistrus* were significantly female-biased during the first year of emergence of both 2005 and 2006 collections at the 450 m site and

also significantly female biased on the second year of emergence of the 2005 collection. At the 1070 m site the populations of both collections did not differ significantly from 50:50. *Z. chionochloae* sex ratios were significantly female-biased during the first year of emergence of the 2005 collection at the 450 m site. The second year of emergence of this collection was significantly male-biased where the third year did not differ from 50:50. The first year of emergence of the 2006 collection at the 1070 m site was significantly male-biased. In all other collections, sites and years of emergence, sex ratio did not differ significantly from 50:50 (Table 7.1).

Table 7.1 Summary of proportional χ2 test for sex ratio deviations from 50:50 by the three insects during three or two years (2005 and 2006 collections respectively) over two elevations. Analysis assumes that each individual is an independent sample. d.f. for all rows equals 1. Bonferroni correction was used, therefore p-values are considered significant if they are <0.017. Significant results are in bold.

Species	Site	Collection	Year of emergence	prop ♀	*n*	χ^2	*P*
E. chionochloae	450 m	2005	2005/06	0.641	902	70.963	**<0.001**
			2006/07	0.596	2870*	105.784	**<0.001**
			2007/08	0.599	217*	8.129	**0.004**
		2006	2006/07	0.632	1574	109.419	**<0.001**
			2007/08	0.618	385	21.039	**<0.001**
	1070 m	2005	2005/06	0.584	269	7.197	**0.007**
			2006/07	0.719	107	19.776	**<0.001**
			2007/08	0.533	30	0.033	0.855
		2006	2006/07	0.574	87	1.655	0.198
			2007/08	0.681	314	40.666	**<0.001**
Gastrancistrus	450 m	2005	2005/06	0.609	361	16.853	**<0.001**
			2006/07	0.570	605*	11.663	**<0.001**
			2007/08	0.400	15*	0.266	0.606
		2006	2006/07	0.592	233	7.571	**0.006**
			2007/08	0.485	66	0.015	0.902
	1070 m	2005	2005/06	0.556	187	2.139	0.144
			2006/07	0.520	25	0	1.000
			2007/08	0.464	28	0.036	0.850
		2006	2006/07	0.470	17	0	1.000
			2007/08	0.479	96	0.009	0.759
Z. chionochloae	450 m	2005	2005/06	0.602	855	35.410	**<0.001**
			2006/07	0.436	493*	7.797	**0.005**
			2007/08	0.571	7*	0	1.000
		2006	2006/07	0.515	1205	1.075	0.300
			2007/08	0.428	56	0.875	0.350
	1070 m	2005	2005/06	0.545	33	0.121	0.728
			2006/07	0.483	29	0	1.000
			2007/08	0.500	5	0.8	0.371
		2006	2006/07	0.357	56	4.018	0.045
			2007/08	0.615	65	3.015	0.082

*numbers are corrected for dissections

7.3.2 Sex ratios and diapause

The proportion of *E. chionochloae* females between the three years of emergence (2005 collection) was not significant in the 450 m site (χ^2 = 5.78, P = 0.0556, n = 3989) with ≥60% females in all three years. However, it was significantly different at the 1070 m site (χ^2 = 6.91, P = 0.0315, n = 406) with very high female percentage during year 2 (72%) and lower female percentages during year 1 (58%) and 3 (53%). The proportion of *E. chionochloae* females from the 2006 collection did not change significantly between years at the 450 m site (χ^2 = 0.26, P = 0.6110, n = 1959) (≥62%) and it was marginally non-significant at the 1070 m site (χ^2 = 3.46, P = 0.0630, n = 401) with 57% females in year 1 and 68% in year 2 (Table 7.1). At the 1070 m site,

females of both collections had higher percentage emergence during the second year, rather than the first year of emergence as was expected.

The proportion of *Gastrancistrus* females of the 2005 collection did not change significantly between the different years at both the 450 m ($\chi^2 = 3.50$, $P = 0.1736$, $n = 981$) or the 1070 m ($\chi^2 = 0.88$, $P = 0.6438$, $n = 240$) sites, although in both sites it started with a female skewed sex ratio in year 1 and ended in a male skewed sex ratio in year 3 (Table 7.1). Similarly, *Gastrancistrus* females from the 2006 collection did not significantly change their proportion of emergence between years at the 450 m site ($\chi^2 = 2.42$, $P = 0.119$, $n = 299$) or at the 1070 m site ($\chi^2 = 0$, $P = 0.9480$, $n = 113$) (Table 7.1).

The proportion of *Z. chionochloae* females from the 2005 collection at the 450 m site changed significantly between the different years ($\chi^2 = 34.83$, $P < 0.001$, $n = 1355$) where the population changed from female-biased to male-biased between year 1 and 2 and then back to female-biased sex ratio in year 3 (Table 7.1). However, at the 1070 m site female proportion did not change significantly between years ($\chi^2 = 2.09$, $P = 0.3512$, $n = 67$) although the population had a slightly female-skew sex ratio in year 1, but male-skew in year 2, a similar trend to the 450 m site. The proportion of female *Z. chionochloae* from the 2006 collection did not change significantly between the two years of emergence at the 450 m site ($\chi^2 = 1.61$, $P = 0.2041$, $n = 1261$), but again changed from slightly female-biased to male-biased sex ratio. There was an opposite significant trend at the 1070 m site where sex ratio changed significantly between the two years of emergence ($\chi^2 = 8.03$, $P = 0.0046$, $n = 121$), starting with heavily male-biased sex ratio in year 1 to female-biased one in year 2.

7.3.3 Treatments and sex ratios

There was no significant effect of treatments on sex ratio in all insects from the 1070 m site, 2005 collection (analysis not shown).

In Table 7.2 I present data for the 450 m site, 2005 collection. *E. chionochloae* had significantly less female-skewed population in plants treated with warmer temperature. The 2007/08 summer season had a significantly less female-skewed

population and the interaction of 2006/07 flowering year and insects from plants treated with GA_3 had a significantly less female-skewed population. In addition, plants treated with root-pruning, warming, and both the combinations of root pruning and GA_3 and warming and GA_3 had a significantly more female-skewed population in the 2007/08 summer season.

Gastrancistrus sp. had a less female-skewed population when treated with root pruning. Insects from the 2007/08 had less female-skewed population than the other two years of emergence, although insects from plants treated with pruning and GA_3 in this year has a significantly more female-skewed population.

Z. chionochloae had a significantly less female-skewed population when treated with warmer temperatures. The 2006/07 summer season also had a significantly less female-skewed population. However, the interaction of the 2006/07 with plants treated with Ga3, root pruning, warming and warming combined with GA_3 had a significantly more female-skewed population.

One of the applied treatments, PG, increased female ratios of *Z. chionochloae* at the 450 m site, 2006 collection (mean = 0.606, z = 3.701, P > 0.001), and there was significantly higher *Z. chionochloae* female emergence during the second year of emergence (2007/08) at the 1070 m site, 2006 collection (mean = 0.826, z = 2.508, P = 0.012).

Table 7.2 Mean proportion of female ratios (± Confidence Intervals (CI) 95%), z and P values for the 450 m site, 2005 collations, from Mixed Effect Models run on sex ratio per pot with 'treatment', 'year of emergence' and their interactions as fixed variables, and 'plant' is a random effect, using binomial distribution. Data is corrected for dissections (data of year 2 and 3 are multiplied by 2, see text. Significant values are in bold.

Species			Mean	Low CI	High CI	z	Pr(>|z|)
E. chionochloae	treatment	C	0.654	0.543	0.751		
		G	0.705	0.599	0.792	0.687	0.492
		P	0.533	0.416	0.646	-1.504	0.133
		PG	0.647	0.537	0.744	-0.093	0.926
		W	0.494	0.380	0.608	-1.979	**0.048**
		WG	0.544	0.414	0.668	-1.293	0.196
	Year emerged	2006/07	0.450	0.367	0.536	-1.149	0.251
		2007/08	0.274	0.206	0.354	-5.110	**<0.001**
	Treatment: year emerged	G: 2006/07	0.357	0.255	0.474	-2.389	**0.017**
		P: 2006/07	0.613	0.493	0.721	1.848	0.065
		PG: 2006/07	0.402	0.290	0.525	-1.577	0.115
		W: 2006/07	0.575	0.458	0.685	1.260	0.208
		WG:2006/07	0.544	0.414	0.669	0.662	0.508
		G: 2007/08	0.660	0.492	0.796	1.872	0.061
		P: 2007/08	0.715	0.564	0.830	2.725	**0.006**
		PG: 2007/08	0.821	0.687	0.905	4.059	**<0.001**
		W: 2007/08	0.690	0.545	0.806	2.533	**0.011**
		WG:2007/08	0.791	0.678	0.872	4.471	**<0.001**
Gastrancistrus	treatment	C	0.698	0.556	0.810		
		G	0.316	0.171	0.509	-1.882	0.060
		P	0.265	0.128	0.470	-2.228	**0.026**
		PG	0.366	0.197	0.576	-1.259	0.208
		W	0.349	0.190	0.552	-1.469	0.142
		WG	0.379	0.204	0.592	-1.121	0.262
	Year emerged	2006/07	0.410	0.269	0.567	-1.125	0.261
		2007/08	0.178	0.080	0.350	-3.301	**0.001**
	Treatment: year emerged	G: 2006/07	0.633	0.437	0.793	1.341	0.180
		P: 2006/07	0.654	0.424	0.829	1.329	0.184
		PG: 2006/07	0.535	0.330	0.729	0.324	0.746
		W: 2006/07	0.615	0.421	0.778	1.169	0.242
		WG:2006/07	0.519	0.308	0.724	0.171	0.864
		G: 2007/08	0.876	0.671	0.960	3.093	**0.002**
		P: 2007/08	0.846	0.570	0.958	2.357	**0.018**
		PG: 2007/08	0.790	0.494	0.936	1.932	0.053
		W: 2007/08	0.651	0.348	0.867	0.978	0.328
		WG:2007/08	0.692	0.403	0.882	1.321	0.187
Z. chionochloae	treatment	C	0.637	0.545	0.720		
		G	0.557	0. 469	0.642	-1.265	0.206
		P	0.517	0.412	0.620	-1.708	0.088
		PG	0.582	0.484	0.674	-0.829	0.407
		W	0.443	0.355	0.534	-2.940	**0.003**
		WG	0.526	0.415	0.635	-1.527	0.127
	Year emerged	2006/07	0.306	0.232	0.391	-4.288	**<0.001**
		2007/08	0.384	0.200	0.607	-1.025	0.306
	Treatment: year emerged	G: 2006/07	0.704	0.585	0.800	3.252	**0.001**
		P: 2006/07	0.778	0.642	0.873	3.670	**0.000**
		PG: 2006/07	0.589	0.443	0.721	1.199	0.231
		W: 2006/07	0.683	0.552	0.791	2.693	**0.007**
		WG:2006/07	0.732	0.599	0.833	3.274	**0.001**
		G: 2007/08	0.587	0.287	0.834	0.549	0.583
		P: 2007/08	0.600	0.283	0.851	0.596	0.551
		PG: 2007/08	0.559	0.259	0.821	0.359	0.719
		W: 2007/08	0.644	0.334	0.867	0.909	0.363
		WG:2007/08	0.610	0.325	0.836	0.746	0.456

7.3.4 Emergence patterns

All three species at the 450 m site showed protandry. That is, median time of overall numbers, show that males were ahead of females in all three species (Figure 7.1), usually by about a week. In the tails (early and late emergence), the pattern of emergence is not as clear as the median emergence. Specifically the wasp species are less clear, with *Z. chionochloae* males appearing to have a late burst of emergence in both years, but that may be misleading due to low numbers of adults emerging.

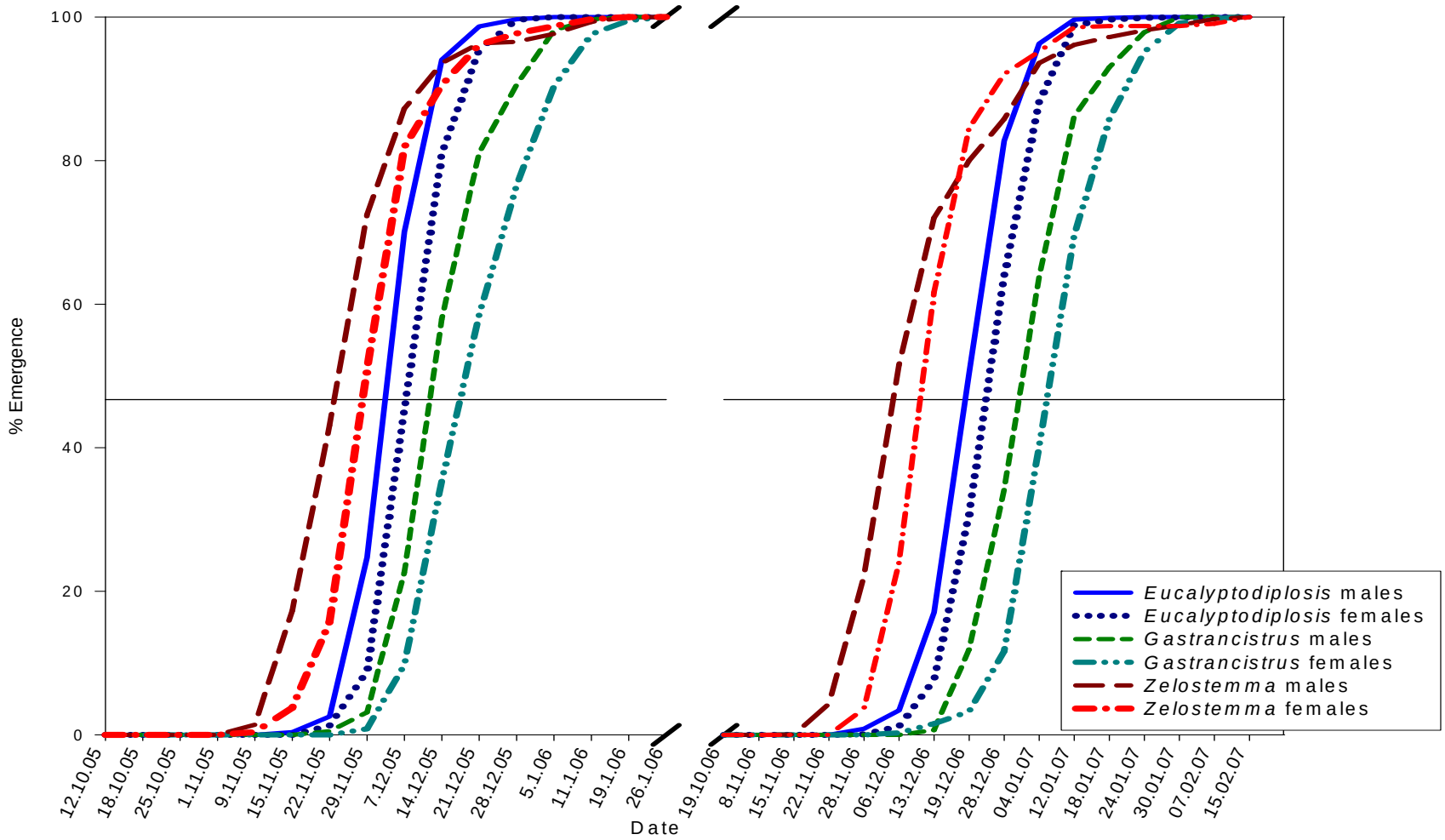

Figure 7.1 Cumulative emergence (%) for males and females of *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae*, in the 2006 (a) and 2007 (b) summer seasons, 2005 collection at 450 m site.

7.4 Discussion

7.4.1 Sex ratio and diapause

My first hypothesis which predicted that more males will undergo prolonged diapause than females and therefore the sex ratios will be less female-skewed with increased diapause length has proved to be wrong.

E. chionochloae had female-biased populations even after three years in diapause in both elevations and collections. Therefore I suggest that *E. chionochloae* females are as successful as males also after several years in diapause. Similarly, *Gastrancistrus* males were not more successful than females in surviving long periods of diapause. However, at the 450 m site, 2005 collection, sex ratio of *Z. chionochloae* changed from significantly female-skewed in year 1 to significantly male-skewed in year 2. Although not significant, the same trend happened in the 1070 m site of the same collection. That could suggest that males are more successful than females in surviving long periods of diapause. However, the 2006 collection, 1070 m site has a significant-opposite trend. Significantly more males came out in the first year than females, but during the second year of emergence, the sex ratio turned to be marginally non-significant female-biased. Therefore I suggest that the difference in sex ratio between the years and collections is probably a result of other factors rather than length of diapause. Such factors can be external signals from the environment or the host, genetic factors and mother's choice whether to have males or females (in the parasitoid species) (see below).

7.4.2 Treatments and sex ratios

My second hypothesis was that females are better predictors than males and could detect a high flowering year better than males and therefore emerge in higher numbers. This could reduce the chances they will miss a "good" year and keep on diapausing. Longer length of diapause may reduce the number of eggs they can produce and therefore decrease their fitness. I therefore expected more female-skewed population across the different plant treatments in comparison to the Control group in all three species.

E. chionochloae females from the 450 m site, 2005 collection either decreased or had no change in their emergence rates across the different treatments in years 1 and 2. In year 3 however, percentage emergence increased across the different treatments (excluding the GA_3

treatment). There was no significant change in sex ratio accross plant treatments in the1070 m site at both the 2005 and 2006 collections. The inconsistency of emergence pattern across the different treatments and years and the positive effect on increasing female-skew sex ratios in year 3, which occurred three years after I have applied the treatments to the plants, suggest that there are other factors which affect the sex ratios of *E. chionochloae* (see below).

Gastrancistrus females of the 2005 collection 450 m site, decreased its female ratios as a response to the pruning treatment. In 2007/08, the third year of emergence after treatments were applied, there was a female-skewed sex ratio of *Gastrancistrus* emerging from the GA_3 and Pruning treatments. This contradicts my hypothesis, suggesting that female *Gastrancistrus* can not better predict external conditions than males and that those females decide whether they should emerge from prolonged diapause or not according to other factors, which may not be different than the factors affecting male emergence.

Z. chionochloae females of the 2005 collection reduced their emergence rates as a response to the warming treatments during year 1, but increased their emergence rate during year 2 and 3 as a response to different treatments. They also responded to plant manipulation in year 1 of the 2006 collection, increasing their female emergence rate as a response to the combination of root pruning and GA_3. Here again, there was no consistency in treatments affecting female emergence.

For all three species, I expected that the treatments will increase female-bias sex ratio in the first year of emergence, as it was closest to the applied treatment, and signals were given to the feeding larvae, while they were still in contact with the plant. Insects which were in prolonged diapause and away from their host plant for more than two years had probably received other signals from the environment or had different reasons for their emergence in those years.

The decision whether to emerge or not in a particular season may be similar for both males and females in all three species and may be related to the oviposition patterns of their mothers, their host quality or genetic factors (see below).

7.4.3 Oviposition patterns and host choice

Some cecidomyiid species are monogenous (individual females have entirely single-sexed broods) (Stuart & Hatchett, 1991; Dorchin et al., 2007). Other cecidomyiid species produce bisexual progenies and occasionally cecidomyiid species produce both unisexual broods (monogenous) and mixed broods (male and female progeny), which are usually dominated by one of the sexes (Stuart & Hatchett, 1991). The Hessian fly, *Mayetiola destructor* (Diptera: Cecidomyiidae) is an example of a species which produces both monogenous and bisexual progeny (Stuart & Hatchett, 1991). The sex ratio in this species is influenced both by genetic factors which are not controlled by the parents and by environmental factors and the habitat the parents are adapted to (Stuart & Hatchett, 1991).

Host choice of *E. chionochloae* females is not known, nor is the pattern of oviposition they perform (i.e. whether females oviposit many eggs on one inflorescence or few eggs on many inflorescences, whether they oviposit all their eggs on the same plant or on different plants etc.). Therefore at this stage it is not known whether larvae on the same plant are all siblings or not. If *E. chionochloae* are monogenous, the determination of population sex ratio involves the relative number of female-producing and male-producing females found within the population and whether some females are producing bisexual progeny as well as monogenous progeny and if they do, what are the percentages of these bisexual producing female relatively to the monogenous producing females. In order to directly test whether each female produces single-sex broods, one would have to mate females in the laboratory with some uninfected plant material and keep each female's progeny in a different container to overwinter. If environmental factors and the habitat the parents are adapted to affecting the sex ratio of the offspring, as well as genetic factors, similar to the findings of Stuart and Hutchett (1991), then my plant treatments may explain in part the differences in sex ratio of *E. chionochloae*. However, it is not known specifically which environmental factor is controlling the sex ratio of the offspring, whether any of my plant and environmental treatments played any role in changing the sex ratio, whether secondary effects of these plant manipulations (i.e., increased humidity in the warmed plants, decreased root-herbivory in the root pruning plants), or whether other cues which I did not control for (e.g., photoperiod, plant quality, plant density) had a significant effect on sex-ratio. Additionally, it is not known whether genetic factors have stronger effect on sex ratio then environmental effects. Furthermore, the number of different females that oviposit on one plant is unknown. Also, different females may lay high or low quality eggs as a result of their own feeding quality. If different females with different sex ratio oviposited on the same plant and if one sex suffered higher larval mortality than the

other, the sex ratio of emerging adults will be affected but the treatments applied to the plants will not contribute to this skew in sex ratio.

Fisher (1930) suggested that breeding populations should have equal parental investment in male and female offspring. Therefore it is expected that monogenous species should also invest equally in male or female broods. However, many species such as *E. chionochloae* have biased sex ratios that are different from 50:50 and investment was found to differ in male and female offspring. Many studies found a deviation from Fisher (1930) prediction which was selected to increase parental fitness. The reasons for these deviations were found to relate to local resource competition (Clark, 1978; Silk, 1983), local resource enhancement (Schwarz, 1988; Stark, 1992), partially overlapping generations (Tepedino & Parker, 1988), conflict between mates (Brockmann & Grafen, 1989) and maternal size (Sugiura & Maeta, 1989). Resource levels were also found to play a significant role in sex allocation in hymenoptera. Haplo-diploid organisms such as Hymenoptera use known mechanisms to determine sex ratio (fertilized eggs which create females and unfertilized eggs which create males) (Cook & Crozier, 1995). The choice of the sex of the brood is made by the female and may depend on the time and energy necessary to produce each of the sexes. Usually female offspring in arthropods are larger in size than males (O'Neill, 1985; Hurlbutt, 1987; Head, 1995; Fairbairn, 1997; Peterson & Roitberg, 2006; Blanckenhorn et al., 2007; Shreeves & Field, 2008) and therefore may be more expensive to produce (Rosenheim et al., 1996). Previous studies found that in populations of hymenopteran bees, high resource levels result in higher average number of cells per nest (Minckley et al., 1994; Peterson & Roitberg, 2006) and a larger proportion of the more expensive sex (Rosenheim et al., 1996). Therefore, if resources are limited, females may produce more of the less expensive sex (usually males) to maximize the number of offspring they produce and increase their fitness. This study started in early summer, during or slightly after females of *E. chionochloae*, *Gastrancistrus* and *Z. chionochloae* have oviposited their eggs in the florets or host larvae. That means sex ratio of the progeny was already determined by other factors (e.g. environmental, maternal, genetic) which I had no control of. Females of the 2005 collection mated and laid eggs at the beginning of a moderate flowering year (2004/05), whereas females of the 2006 collection mated and laid eggs at the beginning of a very high flowering year (2005/06) (Kelly et al., 2008). These initial conditions may have influenced the sex ratio of the hymenopteran species differently in each collection having more male offspring in the 2005 collections and more female offspring in the 2006 collection.

Reproductive success of females may be alternatively limited by the number of fertilized eggs they can produce in their lifetime rather then resource availability (Rosenheim, 1999; Rosenheim et al., 1996). In that case, females would be selected to have female offspring to maximize their fitness (Rosenheim, 1999; Rosenheim et al., 1996).

In order to increase the success of their offspring, parasitoid mothers must consider host quality when ovipositing their progeny. Their preferences may be related to host size (Bai et al., 1992; Mousseau & Fox, 1998), nutritional content (Hastings, 1986) and other host qualities (Thompson & Hagen, 1999 and references therein). Adult *E. chionochloae* females are larger in size than males (Kolesik et al., 2007; pers. obs.) and possibly larvae of females are larger than males too. Therefore, females may represent a better food source for *Z. chionochloae* and *Gastrancistrus* larvae than males. However, the female-biased population of *E. chionochloae* may suggest otherwise. There are some implications of host size to parasitoid progeny sex ratios, i.e., females would prefer to lay their more expensive offspring (usually females) in larger hosts (Charnov et al., 1981; King, 1987and references therein). Host discrimination in *Z. chionochloae* and *Gastrancistrus* is yet to be examined and can benefit from more study.

7.4.4 Emergence patterns

The emergence of males is selected to occur at a time where mates are available. Females however, not only should synchronize their mating with the time males are present but also the timing when the host plant is suitable for ovipositing (Fagerstrom & Wiklund, 1982). Fagerstrom and Wiklund (1982) referred to phytophagous insects but this is probably true for parasitoids as well.

E. chionochloae larvae possibly use photoperiod to enter or terminate diapause at the right time, but in these experiments I did not control for photoperiod and all treatments had the same, natural conditions of photoperiod. The parasitoid species may have also responded to photoperiod for proportion of emergence of males and females. The timing of entering diapause in these parasitoid wasps may be controlled by external cues, the same as for free-living insects but also may be influenced by the host's physiological state or a combination of external cues and host physiology (Tauber et al., 1986). The median emergence of all species was earlier in males than in females (Figure 7.1). The tails of both years (early and late season) were less clear for the parasitoid species in terms of earlier emergence of males than

females. Specifically some *Z. chionochloae* males continued emerging after most females had already emerged at the end of the season; however only a small number of males came out after female emergence had been completed. Nevertheless this pattern may be a result of very low numbers of insects emerging during early and late season. In Chapters 2 and 4, it was seen that *Z. chionochloae* emerged before *E. chionochloae* while *Gastrancistrus* sp. emerged after *E. chionochloae*, which may suggest that the parasitoids use primarily external cues (e.g., temperature, photoperiod) for entering diapause and emergence. Protandry is shown for all species in both years of emergence. That is, diapause did not change the pattern of protandry such as in other studies (i.e, Takeda & Chippendale, 1982; Brockmann, 2004).

To conclude, *E. chionochloae* has female-biased sex ratio in different populations across elevations and after prolonged diapause. There is week evidence that both parasitoid species are female-biased in the first emergence year and male-biased after more than one year in diapause. Therefore I suggest that diapause is not more costly for females of *E. chionochloae* and its parasitoid than for males. Sex ratios of all three species were probably affected by other factors which I did not control for rather than the applied treatments to the plants. Moreover, females of all three species were not found to be better predictors (i.e, more likely to respond to treatments by not entering extended diapause) than males. *E. chionochloae Gastrancistrus* sp. and *Z. chionochloae* all show protandry with males emerging slightly earlier in each season than females.

8. Final Conclusions - Global change implications on the ecological system

For more than ten years, scientists have predicted global mean warming of between 1 and 3˚C in the next century (Mitchell et al., 1995; Kattenberg et al., 1996) with 0.1 to 0.2˚C per decade (IPCC, 2001). Recent modelling supports this theory and predicts that the environment will keep warming (Smith et al., 2007). Numerous studies suggest global change alters current and future species and ecosystems (McCarty, 2001).

8.1 Change in phenology

Variation in the timing of breeding is largely influenced by temperature and precipitation (Crick & Sparks, 1999; Newman, 2005). Many studies found earlier timing of breeding in different species and taxa. For example, plants (Bradley et al., 1999; Parmesan, 2007), insects (Sparks & Carey, 1995; Sparks & Yates, 1997), amphibians (Parmesan, 2007), birds (Crick et al., 1997; McCarty, 2001) and also changes in timing of plant - animal interactions (Sparks & Carey, 1995; Sparks & Yates, 1997; Memmott et al., 2007; Parmesan, 2007). All these studies attributed the earlier breeding and reproduction timing to climate change. Memmott et al., (2007) stated that the phenology of insect-plant interactions may become desynchronized because of global warming. They simulated phenological differences of plant-pollinator systems over 32 years, using a dataset of 429 plant species and their interactions with 1419 species of pollinator insects and one species of pollinator bird. They suggested that specialized pollinators are most vulnerable to these climate changes and therefore to phenological changes and predicted local extinctions due to the lack of overlap in the timing of plant flowering and pollinator activity. According to their study generalist pollinators will be affected as well by having fewer plant species to feed on and greater variation in food supply. On the other hand, plants will suffer lower recruitment and therefore lower growth rates of the populations.

Some studies however, did not find an effect of global warming on the synchrony of host - plant interactions. For example, Buse and Good (1996) studied development acceleration of winter moth (*Operophtera brumata* L.) and its plant host oak (*Quercus robur* L.) in relation to global warming and found that in both species the timing of development had advanced but the synchrony between the two was not affected. However, another more recent study found that hatching of the winter moth was more advanced than oak budburst and therefore first

instars may either starve or feed on older plant material which produces smaller females that produce less eggs and therefore damage their fitness (Visser & Both, 2005).

In Chapters 4 and 5, I discussed the phenology of *Chionochloa* seed predators and the seed predator's parasitoids. Each of these species responds to some environmental factors and synchronized their reproduction timing to that of their host. *Chionochloa* spp. are known to synchronize with each other according to the previous year's summer temperature (Norton & Kelly, 1988; Kelly et al., 1992; Kelly, 1994; Kelly & Sullivan, 1997; Kelly et al., 2000; Kelly & Sork, 2002) and the current year's temperature (Kelly et al., 2008). *M. calamogonus* and *D. similis* emerge relatively early in the season and are highly synchronized with *Chionochloa* flowering (Chapter 4). *E. chionochloae* not only synchronizes its emergence with the timing of *Chionochloa* flowering during the summer season but should also synchronize the year of emergence (or diapause) in order to better exploit its host (Chapters 4 and 6). Moreover, all these seed/flower predators have parasitoids which must synchronize with their hosts and with each other (for mate finding, and to reduce inter-specific competition) in order to maximize their fitness. In Chapter 5 I discussed *E. chionochloae* emergence dates and its two specific parasitoids, *Gastrancistrus* sp. and *Z. chionochloae*. The timing of emergence in these two species is crucial for their survival as these two parasitoids are competing for the same resources and may escape competition or reduce it by attacking *E. chionochloae* at different times.

Males of *E. chionochloae*, *Gastrancistrus* sp. and *Z. chionochloae* usually emerge earlier than females (Chapter 7). This behaviour was probably selected to maximize male fitness by increasing the number of matings they will have in their adult lifetime. Hymenopteran females can reproduce without mating and in that case will have male progeny. However, females in a healthy environment control the sex ratio of their offspring to maximize their fitness. If males do not have a high rate of mating or if the synchrony between males and females of the same species alters, there will be a reduction in fitness for the species.

To summarize, environmental factors, which control reproduction success and survival of species are very important in the synchrony of individuals, populations and communities. If climate change affects these delicate and complex systems the species may not be able to respond to changes quickly enough, fitness reduction will take place and therefore future extinctions may become more likely.

8.2 The role of climate in the ecology of species

Water stress can have a direct effect on species' physiological characteristics such as breeding and seed production. For example, Ross et al., (1985) showed that root pruning increased seed-cone buds of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco). Precipitation levels in general, or more specifically, water stress, are also very important in playing a direct role affecting the distribution of organisms (Woodward, 1987; McCarty, 2001). For example, in northern New Mexico severe drought caused a rapid landscape-scale shift of ponderosa pine forest (*Pinus ponderosa*) and piñon-juniper woodland (*Pinus edulis* and *Juniperus monosperma*) (Allen & Breshears, 1998). *Chionochloa* was not affected by water stress which was applied by root pruning (Turnbull et al., in prep.). However, both *E. chionochloae* and *Z. chionochloae* were found to be sensitive to water stress at the 450 m site (Chapter 6) and had higher emergence rates during the first year of emergence.

Temperature is a major factor, which is known to directly affect physiological characteristics in organisms. As reviewed in Chapter 7 (section 7.1.1), sex ratios of the progeny of females can be influenced by abiotic factors such as temperature, pH, or photoperiod). *E. chionochloae* usually have female-biased sex ratios (Chapters 2, 7), however warmer temperatures in low elevations decrease percentages of female emergence after one or two years in diapause. Similarly, *Z. chionochloae*, a specific hymenopteran parasitoid of *E. chionochloae* had a higher rate of male emergence after one year in diapause in the same altitudes as a result of higher temperatures during its feeding stages (Chapter 7). That may be caused because of higher mortality of females during prolonged diapause, but such cost of diapause for *E. chionochloae* or *Z. chionochloae* females was not proven.

Other studies suggested that high temperatures skew sex ratios. Wilkes (1959) exposed larvae and pupae of the hymenopteran wasp *Dahlbominus fuliginosus* to high temperatures and found a difference in the sensitivity of males and females to high temperatures in the different stages of development. Males were more sensitive to high temperatures in their larval stage and females were more sensitive to high temperatures in their pupal stage. Therefore exposing these insects to high temperatures at different stages of their development may cause skewed sex ratios.

Apart from direct effects, climate may have indirect effects on ecosystems. Gworek et al., (2007) found that the structure of Jefferey pines (*Pinus jeffreyi*) in the eastern Sierra Nevada differs across elevations, where most precipitation and most seedling recruitment occurs at

higher elevations. In addition at lower elevations there was more evaporation, higher plant density and therefore more mistletoe infection, which increased the susceptibility to other pathogens and seed predation by vertebrates and invertebrates. That resulted in higher rates of mortality at lower elevations than in higher ones. Gworek et al., (2007) predicted that populations of Jeffrey pines will shift higher in elevation because of these differences in climate conditions.

Many masting species use temperature as a cue for flowering (Brockie, 1986; Ashton et al., 1988; Norton & Kelly, 1988; Sork et al., 1993; Kelly & Sork, 2002), among these *Chionochloa* species (Mark, 1965a, 1965c; Connor, 1966; Mark, 1968; Kelly et al., 1992; Kelly & Sullivan, 1997; McKone et al., 1998; Kelly et al., 2000; Rees et al., 2002). As temperature increases, the cues for flowering in *Chionochloa* may be very frequent.

The evolutionary origin of mast seeding in *Chionochloa* has been extensively studied in the past (Kelly et al., 1992; Sullivan, 1993; Cone, 1995; Kelly & Sullivan, 1997; Kelly et al., 2000; Sullivan & Kelly, 2000; Kelly et al., 2001; McKone et al., 2001; Rees et al., 2002; Kelly et al., 2008) and in this present study (Chapters 2-6). According to all the above studies the benefits of masting in *Chionochloa* comes from satiation of seed and flower predators. Kelly and Sullivan (1997) showed the effect of different CVs (coefficients of variation, see also Chapter 1 section 1.1.1) of floret production in *C. pallens* on percentages of florets predated. According to their model, if the CV of *C. pallens* dropped slightly from 1.8 to 1.5, predation levels would rise from 32 to 53%, moreover if the CV dropped to 1.0, predation levels would reach 69%. In Chapter 4, I have shown that at least one of the *Chionochloa* specialist seed predators (*D. similis*) reduces in numbers in high flowering years, especially at higher elevations where *Chionochloa* CV is extreme. If *Chionochloa* flowering became less variable among years, the relatively small populations of *D. similis* and *M. calamogonus* would increase to levels that would regularly consume a larger proportion of the flower crop. In other words, *Chionochloa* plants would no longer be able to control the number of its predators, many seeds would be lost to predation and plant fitness would be significantly reduced. If numbers of *D. similis* and *M. calamogonus* increase, I expect their specialist parasitoids (Chapter 5) to increase in numbers as well. If the parasitoid numbers increase, there may be a reduction of *D. similis* and *M. calamogonus* numbers, thereby reducing *Chionochloa* seed predation. However, there is little information on the biology or host specificity of these parasitoids to conclude how significant their predation will be on their hosts. However, flowering in *Chionochloa* plants is also dependent on the plant's resources

(Rees et al., 2002) which suggests that several consecutive warm summers will deplete plant resources and there will not be flowering even though the temperature cue was detected. Therefore, if plants will not produce seeds every year because of lack of resources, populations of seed predators will decrease again and will be controlled by resource available to the plants.

The third seed predator, *E. chionochloae*, is using prolonged diapause and can emerge after 3 years (Chapters 6 and 7). In addition, its two specialist parasitoids, *Gastrancistrus* sp. and *Z. chionochloae* use prolonged diapause which may have been selected as an adaptation to the variable abundance of their host. In the long term, increased temperature may affect *Z. chionochloae* by not having enough host larvae to feed on at lower elevations. This may create a significant reduction in population numbers, as *Z. chionochloae* is most abundant in these low-altitude habitats (Chapter 6). However, at higher elevations if warmer temperatures cause *E. chionochloae* to spend fewer years in diapause, *Z. chionochloae* may shift from lower to higher elevations and establish its populations there. In addition, if *Chionochloa* plants are predicted to shift to higher elevations, populations of *E. chionochloae* should follow them and decrease in numbers in lower altitudes. It is not known how many years *Gastrancistrus* sp. can stay in diapause. If *Gastrancistrus* sp. is capable of staying in diapause for many years, and if *E. chionochloae* shifts to higher elevations, then at lower elevations *Gastrancistrus* chances of survival may be smaller. Although higher temperature did not have any effect on *Gastrancistrus* sp. at higher elevations, predict that their population will increase at higher elevations rather than at lower ones because more hosts may be suitable for their offspring survival in these elevations.

Climate has a major impact on the geographical range of many species. For example, Grace et al., (2002) predicted that under the effect of global warming, the boundary zone between trees and shrubs in mountain vegetation, which is called the 'treeline', will undergo a significant change in structure and position. Danby and Hik (2007) suggested that the treelines will advance to higher elevations and latitudes as temperature keeps increasing. McKone et al., (1998) questioned the capability of *Chionochloa* to shift to higher elevations as the long distance seed dispersal of *Chionochloa* is unknown. They also stated that although climate changes have happened in the past with proper plant adaptations, the current climate change may be too fast for plants to shift to cooler habitats. Nonetheless, few studies have examined the distribution and dynamics of alpine shrubs in response to climate change. Sturm et al., (2001) reported that several species of shrubs in the Alaskan tundra area had higher

abundance as a result of warmer temperatures. Danby and Hik (2007) found that the Willow shrub (*Salix* spp.) in Southwest Yukon, Canada, increased its density with elevation.

8.3 The risk of extinction

How quickly species can respond and adapt to global changes is an important question in the prediction of future events (McKone et al., 1998; McCarty, 2001). Many studies found extinction of populations of different taxa as a result of global change. For example Hoegh-Guldberg (1999) studied the mass extinction of coral reef populations in many parts of the world which is related to increased sea water temperatures in the past 20 years. Two populations of the checkerspot butterfly, *Euphydryas editha bayensis* became locally extinct in 1991 and 1998 as a result of increasing variability of extreme precipitations events in San Jose (McLaughlin et al., 2002). Sala et al., (2000) used scenarios of change in biodiversity as a result of global climate change and other human induced factors such as land use. They predicted that the most severe change in biodiversity will occur in grasslands and Mediterranean zones, while alpine and arctic zones were predicted to have the lowest impact simply because they are more extreme and therefore harsher to exotic animals and humans. The survival of *Chionochloa* spp. may depend on their ability to respond to the indirect changes of global change, by shifting to higher elevations and maintaining the high CVs, which keeps their reproductive rates high in mast years.

However, Kelly et al., (2008) found that different species of *Chionochloa* in different elevations had different thresholds for a given flowering effort. They found that at higher elevations the threshold was lower than at lower elevations and that this lowering of threshold was similar to the reduction in mean temperatures found between elevations. These results are important, because it might suggest that *Chionochloa* use plastic local setting of thresholds. If that is the case, then although temperatures will keep increasing in the future, *Chionochloa* may elevate their local threshold and therefore keep their masting behaviour. However, the survival of masting in *Chionochloa* is probably dependent on the time scale that these thresholds can change. If temperatures will increase in a faster rate than the acclimation of these thresholds, masting in *Chionochloa* might break down.

To summarize, temperature and water stress increase may affect all the organisms in this complex system. It is predicted that all these organisms will be selected to shift to higher altitudes, starting with *Chionochloa* plants, their seed/flower predators and the seed/flower

predator's parasitoids. If the change in temperature is too fast for effective upwards migration of *Chionochloa* plants to a higher elevation, and if *Chionochloa* has a cue to flower every year, flowering in *Chionochloa* may be less variable (McKone et al., 1998) and as a consequence the insects would be less food limited. In that case, predation levels by *D. similis* and *M. calamogonus* are predicted to be higher at least initially and may significantly reduce *Chionochloa* chances of successful reproduction. As for *E. chionochloae*, extended diapause will no longer be beneficial or will reduce in frequency as the cues for emergence will occur more frequently. Species may experience striking change in their reproductive behaviour. These changes raise uncertainty regarding the relative winners and losers in this complex chain of natural enemies over future years if global change leads to significant changes in local climates.

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Appendix 1 - Effect of treatments on flowering at Mt Hutt, Matthew Turnbull et al., in preparation.

EFFECT OF TREATMENTS ON FLOWERING AT MT HUTT

MT, 27 July, 2008

Tussocks were tagged at both 450 m and 1070 m sites. Diameter at base and % of tussock with live leaves were measured in 2005, 2007 and 2008 and used to calculate live basal area in cm2. For 2006, live basal area was estimated by interpolation between 2005 and 2007. Flower count was recorded in 2005, 2006, 2007 and 2008 (February). Flower counts were scaled to per dm2 of live tussock for analysis.

Treatments were as follows:

Abbrev	Name	What
C	control	no manipulation
G	gibberellin	GA_3 applied by foliar spray
P	prune roots	spade cut roots around half circumference
PG	prune + GA	prune + GA_3
W	warming	warming tube over summer
WG	warming + GA	warming + GA_3

Over the three years these were applied in sequence differently:

Code	Year 1	Year 2	Year 3	N plants at each site
CCC	C	C	C	10
CCW	C	C	W	5
CGC	C	G	C	5
CPC	C	P	C	5
CPgC	C	PG	C	5
CWC	C	W	C	5
CWgC	C	W	C	5
GCC	G	C	C	5
GGC	G	G	C	5
PCC	P	C	C	5
PgCC	PG	C	C	5
PgPgC	PG	PG	C	5
PPC	P	P	C	5
WCW	W	C	W	5
WgCW	WG	C	W	5
WgWgC	WG	WG	C	5
WWC	W	W	C	5

Analysis performed using glm in two ways:

1. Using a single factor for treatment ("trt") – each treatment level is a single value which encompasses the three years of treatment e.g. CWC = control in yr1, warming in yr2, control in yr3).
2. Separating treatments into the years they were applied and adding these as factors in subsequent years (in addition to adding previous year's flowering as a covariate – e.g. for 2005 (year 1) only yr1 treatment applies, for 2005 (year 2) previous year flowering and both yr1 and yr2 treatments are entered into the model

Analysis is run using a quasipoisson distribution – this allows for the fact that we are using a hybrid of a count variable (flowering) divided by plant area.

Flowering – 450m

2005

1 flrsdm05.glm<-glm(flrsdm05~trt, family=quasipoisson, data=mthutt450.data)
> anova(flrsdm05.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm05

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			64	145.711		
trt	**11**	**50.745**	**53**	**94.966**	**2.4104**	**0.01641 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(flrsdm05.glm)

Call:
glm(formula = flrsdm05 ~ trt, family = quasipoisson, data = mthutt450.data)

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.6781	-0.8917	-0.1875	0.4450	3.4764

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.10526	0.25174	4.390	5.43e-05	***
trtCCW	0.32026	0.39419	0.812	0.42017	
trtGCC	0.22974	0.40510	0.567	0.57302	
trtGGC	0.18673	0.41053	0.455	0.65108	
trtPCC	-0.25511	0.47640	-0.535	0.59455	
trtPGCC	0.43376	0.38146	1.137	0.26061	
trtPGPGC	-0.07564	0.44730	-0.169	0.86636	
trtPPC	0.15887	0.41413	0.384	0.70279	
trtWCW	**0.93596**	**0.33628**	**2.783**	**0.00744**	**
trtWGCW	0.41644	0.38334	1.086	0.28224	
trtWGWGC	**0.94887**	**0.33533**	**2.830**	**0.00657**	**
trtWWC	**1.02059**	**0.33023**	**3.091**	**0.00318**	**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 1.913877)

Null deviance: 145.711 on 64 degrees of freedom
Residual deviance: 94.966 on 53 degrees of freedom
(25 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 5

2 flrsdm05y1.glm<-glm(flrsdm05~y1, family=quasipoisson, data=mthutt450.data)
> anova(flrsdm05y1.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm05

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			64	145.711		
y1	**5**	**41.538**	**59**	**104.174**	**4.4495**	**0.001661 ** **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(flrsdm05y1.glm)

Call:
glm(formula = flrsdm05 ~ y1, family = quasipoisson, data = mthutt450.data)

Deviance Residuals:
   Min   1Q Median   3Q   Max
-2.7774 -0.9107 -0.2813   0.5303   3.3326

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 1.22378   0.19133   6.396 2.82e-08 ***
y1G   0.08995   0.29462   0.305 0.761207
y1P   -0.14537   0.31641 -0.459 0.647617
y1PG   0.09263   0.29439   0.315 0.754127
y1W   0.86065   0.24460   3.519 0.000842 ***
y1WG   0.59916   0.25840   2.319 0.023894 *

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 1.867047)

    Null deviance: 145.71 on 64 degrees of freedom
Residual deviance: 104.17 on 59 degrees of freedom
  (25 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 5

2006

1 flrsdm06.glm<-glm(flrs06dm06~trt, family=quasipoisson, data=mthutt450.data)
> anova(flrsdm06.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrs06dm06

Terms added sequentially (first to last)

     Df Deviance Resid. Df Resid. Dev   F Pr(>F)
NULL   86   226.767
trt 16   78.095   70   148.672 2.2416 0.01095 *

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(flrsdm06.glm,corr=F)

Call:
glm(formula = flrs06dm06 ~ trt, family = quasipoisson, data = mthutt450.data)

Deviance Residuals:
   Min   1Q Median   3Q   Max
-2.7534 -0.9898 -0.1451   0.5500   4.6167

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) 1.49290   0.22120   6.749 3.57e-09 ***
trtCCW   0.40621   0.33782   1.202 0.23324
trtCGC   0.67729   0.31408   2.156 0.03449 *
trtCPC   0.16342   0.36337   0.450 0.65430
trtCPGC   1.16596   0.28183   4.137 9.64e-05 ***
trtCWC   0.67501   0.31426   2.148 0.03518 *
trtCWGC   0.78334   0.30601   2.560 0.01263 *
trtGCC   0.88664   0.31517   2.813 0.00636 **
trtGGC   0.35397   0.34296   1.032 0.30557
trtPCC   -0.05782   0.42254 -0.137 0.89155
trtPGCC   0.64951   0.31630   2.053 0.04376 *
trtPGPGC   0.51127   0.32806   1.558 0.12363
trtPPC   -0.10162   0.39656 -0.256 0.79850
trtWCW   0.34765   0.36788   0.945 0.34791

trtWGCW 0.84505 0.30161 2.802 0.00657 **
trtWGWGC 0.36339 0.34202 1.063 0.29166
trtWWC 0.59152 0.32109 1.842 0.06967 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 2.177424)

Null deviance: 226.77 on 86 degrees of freedom
Residual deviance: 148.67 on 70 degrees of freedom
(3 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 5

2 flrsdm06y2.glm<-glm(flrs06dm06~flrsdm05+y1+y2, family=quasipoisson, data=mthutt450.data)
> anova(flrsdm06y2.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrs06dm06

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)	
NULL			61	117.999			
flrsdm05	**1**	**7.461**	**60**	**110.538**	**5.0481**	**0.0291**	*****
y1	**5**	**21.760**	**55**	**88.778**	**2.9446**	**0.0209**	*****
y2	5	12.846	50	75.932	1.7384	0.1431	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(flrsdm06y2.glm,corr=F)

Call:
glm(formula = flrs06dm06 ~ flrsdm05 + y1 + y2, family = quasipoisson,
data = mthutt450.data)

Deviance Residuals:

Min	1Q	Median	3Q	Max
-2.3902	-0.7509	-0.2779	0.6342	2.4974

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	1.53467	0.15110	10.157	9.5e-14	***
flrsdm05	**0.03275**	**0.01784**	**1.836**	**0.07226**	**.**
y1G	**0.70439**	**0.23117**	**3.047**	**0.00369**	******
y1P	-0.18415	0.32741	-0.562	0.57632	
y1PG	**0.44931**	**0.23329**	**1.926**	**0.05980**	**.**
y1W	0.01020	0.30095	0.034	0.97310	
y1WG	**0.64306**	**0.22014**	**2.921**	**0.00522**	******
y2G	**-0.51258**	**0.28457**	**-1.801**	**0.07769**	**.**
y2P	-0.07747	0.40234	-0.193	0.84809	
y2PG	-0.07209	0.27577	-0.261	0.79484	
y2W	0.25699	0.30939	0.831	0.41012	
y2WG	**-0.58414**	**0.27861**	**-2.097**	**0.04111**	*****

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 1.477975)

Null deviance: 117.999 on 61 degrees of freedom
Residual deviance: 75.932 on 50 degrees of freedom
(28 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 4

2007
1 flrsdm07.glm<-glm(flrsdm07~trt, family=quasipoisson, data=mthutt450.data)
> anova(flrsdm07.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm07

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			89	271.80		
trt	**16**	**130.71**	**73**	**141.09**	**4.7334**	**1.975e-06 *****

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(flrsdm07.glm,corr=F)

Call:
glm(formula = flrsdm07 ~ trt, family = quasipoisson, data = mthutt450.data)

Deviance Residuals:

Min	1Q	Median	3Q	Max
-3.7587	-1.1314	-0.3024	0.5192	2.5680

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	-1.00239	0.68577	-1.462	0.148116	
trtCCW	0.90808	0.92175	0.985	0.327791	
trtCGC	**1.47365**	**0.82811**	**1.780**	**0.079314**	**.**
trtCPC	0.55611	1.00481	0.553	0.581651	
trtCPGC	**2.09967**	**0.76518**	**2.744**	**0.007633**	****
trtCWC	0.77675	0.95019	0.817	0.416321	
trtCWGC	**2.95740**	**0.72052**	**4.105**	**0.000104**	*****
trtGCC	-2.90963	4.21067	-0.691	0.491749	
trtGGC	**1.94863**	**0.77736**	**2.507**	**0.014412**	****
trtPCC	0.05048	1.16814	0.043	0.965652	
trtPGCC	-0.20827	1.27618	-0.163	0.870815	
trtPGPGC	0.76414	0.95307	0.802	0.425293	
trtPPC	0.40091	1.04890	0.382	0.703407	
trtWCW	**1.59018**	**0.81367**	**1.954**	**0.054493**	**.**
trtWGCW	-1.65687	2.32413	-0.713	0.478181	
trtWGWGC	**1.85681**	**0.78560**	**2.364**	**0.020765**	****
trtWWC	0.58384	0.99744	0.585	0.560124	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 1.725949)

Null deviance: 271.80 on 89 degrees of freedom
Residual deviance: 141.09 on 73 degrees of freedom
AIC: NA

Number of Fisher Scoring iterations: 6

2 flrsdm07y3.glm<-glm(flrsdm07~flrs06dm06+y1+y2+y3, family=quasipoisson, data=mthutt450.data)
> anova(flrsdm07y3.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm07

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			86	266.355		
flrs06dm06	1	3.609	85	262.746	1.9423	0.16759
y1	**5**	**19.885**	**80**	**242.861**	**2.1403**	**0.06992 .**
y2	**5**	**95.527**	**75**	**147.334**	**10.2818**	**1.649e-07 *****
y3	1	3.237	74	144.097	1.7420	0.19095

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
> summary(flrsdm07y3.glm,corr=F)

Call:
glm(formula = flrsdm07 ~ flrs06dm06 + y1 + y2 + y3, family = quasipoisson,
    data = mthutt450.data)

Deviance Residuals:
   Min      1Q  Median      3Q     Max
-3.6786  -1.1106  -0.4570   0.4631   2.6404

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -1.07154    0.60850  -1.761  0.08238 .
flrs06dm06  -0.01052    0.02519  -0.418  0.67742
y1G          0.18094    0.54512   0.332  0.74089
y1P         -0.17515    0.88637  -0.198  0.84390
y1PG        -1.11427    0.68486  -1.627  0.10799
y1W          0.24966    0.65747   0.380  0.70524
y1WG        -1.23968    0.44975  -2.756  0.00735 **
y2G          1.79122    0.65974   2.715  0.00824 **
y2P          0.68343    0.82780   0.826  0.41168
y2PG         2.24600    0.68372   3.285  0.00156 **
y2W          0.70854    0.85598   0.828  0.41047
y2WG         3.15282    0.62574   5.039 3.22e-06 ***
y3W          1.02908    0.78116   1.317  0.19178
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 1.858171)

    Null deviance: 266.36  on 86  degrees of freedom
Residual deviance: 144.10  on 74  degrees of freedom
  (3 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 6
```

2008

1 flrsdm08.glm<-glm(flrsdm08~trt, family=quasipoisson, data=mthutt450.data)

```
> anova(flrsdm08.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm08

Terms added sequentially (first to last)
```

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			87	215.060		
trt	**16**	**123.260**	**71**	**91.801**	**5.9979**	**4.647e-08 *****

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
> summary(flrsdm08.glm,corr=F)

Call:
glm(formula = flrsdm08 ~ trt, family = quasipoisson, data = mthutt450.data)

Deviance Residuals:
   Min      1Q  Median      3Q     Max
-2.7406  -0.7107  -0.1150   0.4199   2.7794

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.94973    0.22290   4.261 6.16e-05 ***
trtCCW       0.28458    0.35277   0.807  0.42253
trtCGC      -3.99675    2.60947  -1.532  0.13006
trtCPC       0.96793    0.31124   3.110  0.00269 **
trtCPGC     -1.79836    0.80614  -2.231  0.02885 *
trtCWC      -0.51794    0.46529  -1.113  0.26939
trtCWGC     -3.58081    1.90193  -1.883  0.06383 .
trtGCC      -0.31633    0.43132  -0.733  0.46573
trtGGC      -1.01800    0.56979  -1.787  0.07826 .
trtPCC      -0.28498    0.42641  -0.668  0.50610
trtPGCC      0.07416    0.37677   0.197  0.84452
trtPGPGC    -3.35767    1.70408  -1.970  0.05270 .
trtPPC       0.36292    0.34469   1.053  0.29597
trtWCW       0.12868    0.37022   0.348  0.72918
trtWGCW      0.21717    0.36009   0.603  0.54836
trtWGWGC    -0.58092    0.47680  -1.218  0.22711
trtWWC       0.72774    0.31254   2.328  0.02274 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 1.284397)

    Null deviance: 215.06  on 87  degrees of freedom
Residual deviance:  91.80  on 71  degrees of freedom
  (2 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 5
```

2 flrsdm08y3.glm<-glm(flrsdm08~flrsdm07+y1+y2+y3, family=quasipoisson, data=mthutt450.data)

```
> anova(flrsdm08y3.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm08

Terms added sequentially (first to last)


        Df Deviance Resid. Df Resid. Dev       F    Pr(>F)
NULL                     87    215.060
flrsdm07  1    3.265        86    211.795  2.3522   0.12932
y1        5   21.522        81    190.273  3.1010   0.01343 *
y2        5   85.286        76    104.987 12.2880 1.033e-08 ***
y3        1    2.280        75    102.707  1.6423   0.20396
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> summary(flrsdm08y3.glm,corr=F)

Call:
glm(formula = flrsdm08 ~ flrsdm07 + y1 + y2 + y3, family = quasipoisson,
    data = mthutt450.data)

Deviance Residuals:
```

```
   Min      1Q  Median      3Q     Max
-2.6938 -0.8624 -0.2833  0.4067  3.0312

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.89152    0.20896   4.266 5.73e-05 ***
flrsdm07     0.16386    0.06756   2.426 0.017692 *
y1G         -0.09331    0.38472  -0.243 0.809031
y1P         -0.43727    0.28047  -1.559 0.123189
y1PG         0.03909    0.37250   0.105 0.916711
y1W          0.62521    0.30691   2.037 0.045166 *
y1WG         0.94209    0.40803   2.309 0.023708 *
y2G         -1.88081    0.61180  -3.074 0.002943 **
y2P          0.83590    0.26976   3.099 0.002736 **
y2PG        -2.67567    0.77969  -3.432 0.000979 ***
y2W         -0.13879    0.35107  -0.395 0.693713
y2WG        -2.62784    0.74736  -3.516 0.000747 ***
y3W         -0.47478    0.37692  -1.260 0.211716
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 1.388115)

    Null deviance: 215.06  on 87  degrees of freedom
Residual deviance: 102.71  on 75  degrees of freedom
  (2 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 6
```

Flowering – 1050m

2005

1 flrsdm05.glm<-glm(flrsdm05~trt, family=quasipoisson, data=mthutt1050.data)

```
> anova(flrsdm05.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm05

Terms added sequentially (first to last)


     Df Deviance Resid. Df Resid. Dev      F Pr(>F)
NULL                    59     32.898
trt  10    8.331        49     24.568 1.6306 0.1258 ns
> summary(flrsdm05.glm,corr=F)

Call:
glm(formula = flrsdm05 ~ trt, family = quasipoisson, data = mthutt1050.data)

Deviance Residuals:
   Min      1Q  Median      3Q     Max
-1.3115 -0.5712 -0.1209  0.2729  1.9885

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.99425    0.37159  -2.676   0.0101 *
trtGCC       0.37807    0.57210   0.661   0.5118
trtGGC      -0.35282    0.72874  -0.484   0.6304
trtPCC      -0.97186    0.93162  -1.043   0.3020
trtPGCC      0.02667    0.63794   0.042   0.9668
trtPGPGC     0.84343    0.50684   1.664   0.1025
trtPPC       0.26028    0.59241   0.439   0.6623
```

```
trtWCW       0.51622    0.55035   0.938   0.3529
trtWGCW     -1.81916    1.35682  -1.341   0.1862
trtWGWGC     0.79580    0.51252   1.553   0.1269
trtWWC      -0.35282    0.72874  -0.484   0.6304
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 0.5108885)

    Null deviance: 32.898  on 59  degrees of freedom
Residual deviance: 24.568  on 49  degrees of freedom
  (30 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 5
```

2 flrsdm05y1.glm<-glm(flrsdm05~y1, family=quasipoisson, data=mthutt1050.data)

```
> anova(flrsdm05y1.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm05

Terms added sequentially (first to last)


     Df Deviance Resid. Df Resid. Dev      F Pr(>F)
NULL                    59     32.898
y1    5    1.223        54     31.675 0.3286 0.8936 ns
> summary(flrsdm05y1.glm,corr=F)

Call:
glm(formula = flrsdm05 ~ y1, family = quasipoisson, data = mthutt1050.data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.1136  -0.6637  -0.2241   0.2376   2.6793

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.99425    0.44853  -2.217   0.0309 *
y1G          0.07796    0.62231   0.125   0.9008
y1P         -0.17693    0.66430  -0.266   0.7910
y1PG         0.51622    0.56678   0.911   0.3665
y1W          0.17327    0.60856   0.285   0.7769
y1WG         0.17327    0.60856   0.285   0.7769
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 0.7443586)

    Null deviance: 32.898  on 59  degrees of freedom
Residual deviance: 31.675  on 54  degrees of freedom
  (30 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 6
```

2006

1 flrsdm06.glm<-glm(flrsdm06corr~trt, family=quasipoisson, data=mthutt1050.data)

```
> anova(flrsdm06.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log
```

Response: flrsdm06corr

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			82	200.277		
trt	**15**	**90.017**	**67**	**110.260**	**4.2563**	**1.84e-05 *****

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
> summary(flrsdm06.glm,corr=F)

Call:
glm(formula = flrsdm06corr ~ trt, family = quasipoisson, data = mthutt1050.data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.98212  -0.68183   0.05799   0.63760   3.06227

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.48299    0.17889   8.290 7.22e-12 ***
trtCGC       0.45863    0.26918   1.704  0.09305 .
trtCPC      -0.08371    0.31873  -0.263  0.79364
trtCPGC     -0.05365    0.31548  -0.170  0.86547
trtCWC       0.22477    0.30967   0.726  0.47047
trtCWGC      0.68866    0.25327   2.719  0.00833 **
trtGCC       0.57320    0.26092   2.197  0.03149 *
trtGGC       0.50908    0.26546   1.918  0.05941 .
trtPCC      -0.65521    0.39400  -1.663  0.10099
trtPGCC      0.73230    0.25054   2.923  0.00473 **
trtPGPGC     0.07221    0.30256   0.239  0.81209
trtPPC      -0.89821    0.43489  -2.065  0.04276 *
trtWCW      -0.39127    0.38770  -1.009  0.31650
trtWGCW      0.19560    0.29091   0.672  0.50365
trtWGWGC    -0.37192    0.35332  -1.053  0.29628
trtWWC      -1.00199    0.45422  -2.206  0.03082 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 1.409956)

    Null deviance: 200.28  on 82  degrees of freedom
Residual deviance: 110.26  on 67  degrees of freedom
  (7 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 5
```

2 flrsdm06y2.glm<-glm(flrsdm06corr~flrsdm05+y1+y2, family=quasipoisson, data=mthutt1050.data)

```
> anova(flrsdm06y2.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm06corr

Terms added sequentially (first to last)
```

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			58	116.947		
flrsdm05	1	2.269	57	114.678	2.8308	0.099104 .
y1	**5**	**56.798**	**52**	**57.880**	**14.1702**	**1.865e-08 *****
y2	**5**	**16.138**	**47**	**41.742**	**4.0263**	**0.004018 ****

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```
> summary(flrsdm06y2.glm,corr=F)

Call:
glm(formula = flrsdm06corr ~ flrsdm05 + y1 + y2, family = quasipoisson,
    data = mthutt1050.data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-2.4610  -0.5429  -0.1095   0.6057   1.4263

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  1.38520    0.14200   9.755  7.1e-13 ***
flrsdm05     0.25363    0.11061   2.293 0.026366 *
y1G          0.52516    0.19809   2.651 0.010901 *
y1P         -0.59327    0.29845  -1.988 0.052670 .
y1PG         0.73131    0.18892   3.871 0.000333 ***
y1W         -0.43719    0.29302  -1.492 0.142382
y1WG         0.27770    0.22251   1.248 0.218196
y2G          0.01316    0.20908   0.063 0.950061
y2P         -0.34006    0.40204  -0.846 0.401931
y2PG        -0.78775    0.23395  -3.367 0.001523 **
y2W         -0.53406    0.40937  -1.305 0.198388
y2WG        -0.81216    0.31647  -2.566 0.013524 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 0.8016481)

    Null deviance: 116.947  on 58  degrees of freedom
Residual deviance:  41.742  on 47  degrees of freedom
  (31 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 4
```

2007

1 flrsdm07.glm<-glm(flrsdm07~trt, family=quasipoisson, data=mthutt1050.data)

```
> anova(flrsdm07.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm07

Terms added sequentially (first to last)


     Df Deviance Resid. Df Resid. Dev      F    Pr(>F)
NULL                    89     61.990
trt  16   38.221        73     23.768 6.9459 2.514e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(flrsdm07.glm,corr=F)

Call:
glm(formula = flrsdm07 ~ trt, family = quasipoisson, data = mthutt1050.data)

Deviance Residuals:
      Min         1Q      Median         3Q        Max
-1.779e+00  -2.821e-01  -6.949e-02  -5.519e-05   2.460e+00

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)   -4.61216    1.86100  -2.478  0.01551 *
trtCCW       -15.69043 4076.12993  -0.004  0.99694
```

```
trtCGC        2.84502    1.96621   1.447  0.15219
trtCPC      -15.69043 4076.12993  -0.004  0.99694
trtCPGC       2.60476    1.99383   1.306  0.19551
trtCWC        0.05105    3.16942   0.016  0.98719
trtCWGC       5.07056    1.87265   2.708  0.00843 **
trtGCC      -15.69043 4076.12993  -0.004  0.99694
trtGGC      -15.69043 4076.12993  -0.004  0.99694
trtPCC        1.88609    2.12458   0.888  0.37759
trtPGCC       1.61759    2.19941   0.735  0.46441
trtPGPGC      3.66935    1.90785   1.923  0.05834 .
trtPPC        2.26559    2.04502   1.108  0.27156
trtWCW        3.38327    1.92312   1.759  0.08272 .
trtWGCW     -15.69043 4076.12993  -0.004  0.99694
trtWGWGC      1.38798    2.27861   0.609  0.54433
trtWWC       -0.02316    3.24842  -0.007  0.99433
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for quasipoisson family taken to be 0.3439218)

    Null deviance: 61.990  on 89  degrees of freedom
Residual deviance: 23.768  on 73  degrees of freedom
AIC: NA

Number of Fisher Scoring iterations: 18

2 flrsdm07y3.glm<-glm(flrsdm07~flrsdm06corr+y1+y2+y3, family=quasipoisson, data=mthutt1050.data)
> anova(flrsdm07y3.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm07

Terms added sequentially (first to last)


             Df Deviance Resid. Df Resid. Dev      F    Pr(>F)
NULL                         82     58.669
flrsdm06corr  1   18.989        81     39.680 75.9993 8.717e-13 ***
y1            5    6.428        76     33.252  5.1452 0.0004466 ***
y2            5   14.605        71     18.647 11.6903 3.136e-08 ***
y3            1    0.107        70     18.540  0.4268 0.5157034
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(flrsdm07y3.glm,corr=F)

Call:
glm(formula = flrsdm07 ~ flrsdm06corr + y1 + y2 + y3, family = quasipoisson,
    data = mthutt1050.data)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.93166  -0.30143  -0.12376   0.01863   1.30648

Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept)    -5.29268    0.98327  -5.383 9.25e-07 ***
flrsdm06corr    0.10863    0.02765   3.929 0.000198 ***
y1G           -16.68243 1316.15738  -0.013 0.989923
y1P             2.44639    1.16260   2.104 0.038951 *
y1PG            1.09546    0.66735   1.641 0.105180
y1W             1.01051    2.52265   0.401 0.689953
y1WG           -2.89089    1.19624  -2.417 0.018274 *
y2G             2.71544    1.10071   2.467 0.016075 *
y2P             0.19500    1.01869   0.191 0.848749
y2PG            2.74318    0.91962   2.983 0.003927 **
```

y2W -0.17716 2.52404 -0.070 0.944245
y2WG 4.51161 0.98866 4.563 2.09e-05 *
y3W 2.07578 2.69637 0.770 0.443982

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for quasipoisson family taken to be 0.2498593)

Null deviance: 58.669 on 82 degrees of freedom
Residual deviance: 18.540 on 70 degrees of freedom
(7 observations deleted due to missingness)
AIC: NA

Number of Fisher Scoring iterations: 17

2008

1 flrsdm08.glm<-glm(flrsdm08~trt, family=quasipoisson, data=mthutt1050.data)
> anova(flrsdm08.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm08

Terms added sequentially (first to last)

Df Deviance Resid. Df Resid. Dev F Pr(>F)
NULL 89 27.681
trt 16 14.805 73 12.876 4.2218 1.066e-05 *?**

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(flrsdm08.glm,corr=F)

Call:
glm(formula = flrsdm08 ~ trt, family = quasipoisson, data = mthutt1050.data)

Deviance Residuals:
Min 1Q Median 3Q Max
-8.944e-01 -2.030e-05 -2.030e-05 -2.030e-05 1.799e+00

Coefficients:
Estimate Std. Error t value Pr(>|t|)
(Intercept) -2.230e+01 6.255e+03 -0.004 0.997
trtCCW 5.279e-11 1.083e+04 4.87e-15 1.000
trtCGC 5.278e-11 1.083e+04 4.87e-15 1.000
trtCPC 2.069e+01 6.255e+03 0.003 0.997
trtCPGC 5.277e-11 1.083e+04 4.87e-15 1.000
trtCWC 2.139e+01 6.255e+03 0.003 0.997
trtCWGC 5.280e-11 1.083e+04 4.87e-15 1.000
trtGCC 5.279e-11 1.083e+04 4.87e-15 1.000
trtGGC 2.069e+01 6.255e+03 0.003 0.997
trtPCC 5.282e-11 1.083e+04 4.88e-15 1.000
trtPGCC 5.284e-11 1.083e+04 4.88e-15 1.000
trtPGPGC 5.142e-11 1.083e+04 4.75e-15 1.000
trtPPC 5.267e-11 1.083e+04 4.86e-15 1.000
trtWCW 5.320e-11 1.083e+04 4.91e-15 1.000
trtWGCW 5.268e-11 1.083e+04 4.86e-15 1.000
trtWGWGC 5.186e-11 1.083e+04 4.79e-15 1.000
trtWWC 5.260e-11 1.083e+04 4.86e-15 1.000

(Dispersion parameter for quasipoisson family taken to be 0.2191781)

Null deviance: 27.681 on 89 degrees of freedom
Residual deviance: 12.876 on 73 degrees of freedom
AIC: NA

```
Number of Fisher Scoring iterations: 20

2 flrsdm08y3.glm<-glm(flrsdm08~flrsdm07+y1+y2+y3, family=quasipoisson, data=mthutt1050.data)
> anova(flrsdm08y3.glm,test="F")
Analysis of Deviance Table

Model: quasipoisson, link: log

Response: flrsdm08

Terms added sequentially (first to last)


        Df  Deviance Resid. Df Resid. Dev         F    Pr(>F)
NULL                     89    27.6807
flrsdm07  1    2.6034   88    25.0773   14.3186 0.0003035 ***
y1        5    4.5162   83    20.5611    4.9678 0.0005387 ***
y2        5    8.5782   78    11.9829    9.4360 4.791e-07 ***
y3        1 1.760e-09   77    11.9829 9.682e-09 0.9999217
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> summary(flrsdm08y3.glm,corr=F)

Call:
glm(formula = flrsdm08 ~ flrsdm07 + y1 + y2 + y3, family = quasipoisson,
    data = mthutt1050.data)

Deviance Residuals:
      Min         1Q      Median         3Q        Max
-1.000e+00  -1.178e-05  -2.176e-06  -2.107e-08   1.595e+00

Coefficients:
            Estimate Std. Error   t value Pr(>|t|)
(Intercept)   -42.289  15132.752    -0.003    0.998
flrsdm07     -334.020  43155.627    -0.008    0.994
y1G            19.043   7893.622     0.002    0.998
y1P           -21.466  15304.374    -0.001    0.999
y1PG            3.378  98700.830  3.42e-05    1.000
y1W           -23.030  18329.662    -0.001    0.999
y1WG           -2.111  25411.652 -8.31e-05    1.000
y2G            21.636  12910.904     0.002    0.999
y2P            40.679  15132.752     0.003    0.998
y2PG           15.519  98231.004  1.58e-04    1.000
y2W            41.596  15132.752     0.003    0.998
y2WG           20.182  21852.863     0.001    0.999
y3W            18.354  23080.590     0.001    0.999

(Dispersion parameter for quasipoisson family taken to be 0.1818182)

    Null deviance: 27.681  on 89  degrees of freedom
Residual deviance: 11.983  on 77  degrees of freedom
AIC: NA

Number of Fisher Scoring iterations: 23
```